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EDITORIAL

1α25 Dihydroxyvitamin D—A New Tool in Medicine

In 1922 following work at the University of Wisconsin USA McCollum et al (17) identified vitamin D as a factor responsible for the calcification of rachitic bone Later Steenbock and Black (22) from the same university demonstrated the presence of an antirachitic factor in certain lipids after irradiation with ultraviolet light a process that became important for the synthesis of vitamin D For many decades to follow it was assumed that vitamin D exerted its physiological function without being metabolically altered In 1966 however a group in Steenbock s former laboratory directed by DeLuca succeeded in making a radioactive vitamin D preparation with a specific activity that was high enough to allow studies of its metabolism under physiological conditions (18) This was the first step in the development of the exciting new concept of vitamin D where the vitamin is regarded as a prohormone (5) subsequently being metabolized to 1α25(OH) D (13) which constitutes a central regulator of calcium and phosphate homeostasis (3)

The main target organs for 1α25(OH)₂D are the intestine where it regulates the absorption of calcium and promotes the absorption of phosphate and the bone where it permits normal remodeling and a normal response to parathyroid hormone (9) 1α25(OH)₂D has also been found to aid in the reabsorption of calcium in the kidney (6) and recently receptors have been found in parathyroid tissue (1) where 1α25(OH)₂D may have an inhibitory effect (4) In its action 1α25(OH) D resembles a typical steroid hormone (10) which—at least in the intestinal cells—exerts its effect through a hormone receptor complex in the nucleus subsequently inducing the formation of new proteins such as calcium binding protein

The synthesis of 1α25(OH)₂D which occurs in the kidney is promoted by a low concentration of calcium in serum mainly mediated through an increase in the serum level of parathyroid hormone but the production of 1α25(OH)₂D is also increased by a low serum concentration of phosphate (19) An insufficient production of 1α25(OH) D can result in low serum levels of calcium and phosphate secondary hyperparathyroidism impaired mineralization of bone and neuromuscular disturbances

The rationale for treatment with 1α25(OH) D is obvious under conditions involving impaired formation in the kidney such as hereditary vitamin D-dependent rickets where an enzymatic defect seems to lead to a defective synthesis of 1α25(OH) D (7) This defect is well compensated for with exogenously administered 1α25(OH)₂D In uremia the levels of 1α25(OH) D seem to follow the decrease in kidney cell mass In most cases some production of 1α25(OH) D occurs allowing the bone to respond to the increased levels of parathyroid hormone which together with other aberrations occurring in uremia give rise to the complex picture of uremic osteodystrophy In many cases of uremia 1α25(OH) D given in physiological doses is beneficial to the patient although it does not always cure the bone disease (11) In a tubular dysfunction resulting in hypophosphatemia and impaired bone mineralization such as X linked hypophosphatemic rickets 1α25(OH) D might be a useful adjunct to phosphate therapy in order to also increase the calcium absorption and prevent hyperparathyroidism (20)

Much of the clinical picture in hypoparathyroidism and also pseudohypoparathyroidism is caused by the low levels of 1α25(OH) D Since in hypoparathyroidism the specific aim is to restore the serum calcium a physiological dose of 1α25(OH) D together with calcium supplements probably constitutes the best currently known therapy (21)

The production of 1α25(OH) D may also be affected in osteoporosis The common factor in the pathogenesis of the various forms of generalized oesteoporosis has been thought to be a shift in the source of serum calcium from absorption of calcium from the intestine to resorption from the bone resulting in a negative calcium balance In glucocorticoid induced osteoporosis and in senile osteoporosis the intestinal absorption of calcium seems to be reduced (2 8) 1αOHD₃ which is converted to 1α25(OH)₂D₃ in vivo (12) has been shown

to increase calcium absorption in patients treated with low doses of glucocorticoids and also in patients with senile osteoporosis (15) In these conditions therapeutical trials with 1α25(OH) D seem to be justified Finally in postmenopausal osteoporosis the negative calcium balance has been found more improved by estrogen and 1αOHD$_3$ than by estrogen alone (16)

As indicated above 1α25(OH) D or 1αOHD can be an effective therapeutic agent in several diseases An alternative treatment might be the substitution for the active vitamin D metabolite with superphysiological doses of vitamin D However if dosed to give an adequate effect this results in a certain risk of accumulation and the development of toxic symptoms Compared to vitamin D 1α25(OH) D if given in excess will result in more rapidly developing signs of overdosage which will disappear promptly upon the interruption of treatment (14)

The elucidation of the vitamin D endocrine system has provided us with a new concept of the physiology of a vitamin and an understanding of the pathophysiology of several diseases involving calcium and phosphate homeostasis Now we can also exploit the therapeutical consequences of these findings

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REFERENCES

1 Brumbaugh P F Hughes M R & Haussler M R Cytoplasmatic and nuclear binding components of 1α25-dihydroxy vitamin D$_3$ in chick parathyroid glands Proc Natl Acad Sci USA 72 4871 1975

2 Bullamore J R Wilkinson R Gallagher J C Nordin B E C & Marshall D H Effect of age on calcium absorption Lancet 2 535 1970

3 Chen T C Castillo L Korycka Dahl M & DeLuca H F Role of vitamin D metabolites in phosphate transport of rat intestine J Nutr 104 1056 1974

4 Chertow B S Baylink D J Wergedahl J E Su M H H & Norman A W Decrease in serum immunoreactive parathyroid hormone in rats and in parathyroid hormone secretion in vitro by 1 25 dihydroxycholecalciferol J Clin Invest 56 668 1975

5 DeLuca H F Vitamin D The vitamin and the hormone Fed Proc 33 2211 1974

6 – Vitamin D metabolism Clin Endocrinol (Suppl) 1 1977

7 Fraser D Kooh S W Kind H P Holick M F Tanaka Y & DeLuca H F Pathogenesis of hereditary vitamin D dependent rickets An inborn error of vitamin D metabolism involving defective conversion of 25-hydroxy vitamin D to 1α25 dihydroxy vitamin D N Engl J Med 289 817 1973

8 Gallagher J C Aaron J Horsman A Wilkinson R & Nordin B E C Corticosteroid osteoporosis J Clin Endocrinol Metab 2 355 1973

9 Garabedian M Tanaka Y Holick M F & DeLuca H F Response of intestinal calcium transport and bone calcium mobilization to 1 25-dihydroxy vitamin D$_3$ in thyroparathyroidectomized rats Endocrinology 99 1022 1974

10 Haussler M R Vitamin D Mode of action and its biomedical applications Nutr Rev 32 257 1974

11 Henderson R G Russell R G G Ledingham J G G et al Effects of 1 25 dihydroxycholecalciferol on calcium absorption muscle weakness and bone disease in chronic renal failure Lance 1 379 1974

12 Holick M F DeBlanco M C Clarke M B Heuley J W Neer R M DeLuca H F & Potts J T Jr The metabolism of [6-^{3}H] 1α hydroxycholecalciferol to [6-^{3}H] 1α25-dihydroxycholecalciferol in man J Clin Endocrinol Metab 44 595 1977

13 Holick M F Schnoes H F DeLuca H F Suda T & Cousins R J Isolation and identification of 1 25-dihydroxycholecalciferol A metabolite of vitamin D active in intestine Biochemistry 10 2799 1971

14 Kanis J A & Russell R G G Rate of reversal of hypercalcemia and hypercalciuria induced by vitamin D and its 1α hydroxylated derivatives Br Med J 1 78 1977

15 Lindgren U Lindholm S & Sarby B Short term effects of 1 alpha-hydroxy vitamin D$_3$ in patients on corticosteroid treatment and in patients with senile osteoporosis Acta Med Scand 204 89 1978

16 Marshall D H & Nordin B E C The effect of 1α hydroxyvitamin D$_3$ with and without estrogens on calcium balance in post menopausal women Clin Endocrinol (Suppl) 159 1977

17 McCollum E V Simonds N Becker J E & Shipley P G Studies on experimental rickets XXI An experimental demonstration of the existence of a vitamin which promotes calcium deposition Bull Johns Hopkins Hosp 33 229 1922

18 Neville P F & DeLuca H F The synthesis of 1,2 ^{3}H vitamin D$_3$ and the tissue localization of a 0 25 μg (10 IU) dose per rat J Biochem 5 2201 1966

19 Pitt M J & Haussler M R Vitamin D biochemistry and clinical applications Skeletal Radiol 1 191 1977

20 Rasmussen H Anast C Parks J et al 1α(OH)D$_3$ in treatment of hypophosphatemic rickets Clin Res 26 486A 1976

21 Russell R G G Smith R Walton R J et al 1 25-dihydroxycholecalciferol and 1α hydroxycholecalciferol in hypoparathyroidism Lancet 2 14 1974

22 Steenbock H & Black A Fat soluble vitamins XVII The induction of growth promoting and calcifying properties in a ration by exposure to ultraviolet light J Biol Chem 61 405 1924

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Early and Sudden Deaths after Myocardial Infarction

A Report from the Swedish CCU Study

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ABSTRACT 1329 patients were discharged alive after acute myocardial infarction initially treated in a CCU In a five year follow up 537 (40%) of the patients died Routine data registered uniformly during the CCU period showed that apart from age the most important factors regarding long term prognosis in general were previous ischaemic heart disease and direct or indirect signs of heart failure registered in the CCU The possibilities to predict sudden death (130 patients died within 2 hours of onset of final symptoms during the follow up period) were small although a definite dominance of this mode of death was noted in patients below 60 years of age The clinical profile of the majority of the 134 patients who died during the first half year was distinguished by a history of prior myocardial infarction and signs of left heart failure during the CCU stay However in a significant number of patients dying early after discharge, none of the ordinary unfavourable prognostic signs had been registered

Key words myocardial infarction prognosis sudden death

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Using the routine data registered in coronary care units (CCU) prognosis for the next 2 3 years can be evaluated reasonably well in groups of survivors of acute myocardial infarction (AMI) (3 6 11) To prognosticate for longer periods is feasible but preferably requires new patient data accumulated at regular intervals during the follow up Even with such a dynamic approach however prediction of sudden death after AMI has been only modestly successful

The aim of the present report has been to evaluate the predictive power of routine data registered in CCUs with regard to the prognosis for the first half year after the AMI and sudden death as well as to long term prognosis in general

PATIENTS AND METHODS

Twelve Swedish hospitals participated in a collaborative CCU study in 1969 All patients with AMI admitted to the CCUs during that year were included in the study Ordinary criteria for admission and diagnoses were used and have been presented earlier with a description of the early stage and short term prognosis of the patients (8) A uniform record was used with codified data suitable for subsequent transfer to tape and computer analysis

Participating hospitals and main organizers were County Hospital Östersund (I Bergstrom) the Academic Hospital Uppsala (I Cullhed) St Gorans Hospital Stockholm (H Eliasch) County Hospital Borås (S Å Forsberg) Sahlgrenska Hospital Gothenburg (R Henning) General Hospital Malmo (B W Johansson) County Hospital Falun (M Korsgren) County Hospital Vanersborg (T Leonhardt) Serafimerlasarettet Stockholm (T Lundman) County Hospital Uddevalla (R Malmcrona) Karolinska Hospital Stockholm (L Oro) University Hospital Linkoping (J Sievers)

There were 1329 patients with a mean age of 65 years discharged alive after an AMI The range and sex distribution is shown in Fig 1 The follow up period was 4–5 years and ended on Dec 31 1973 Treatment to individual patients was given according to local routines and was not centrally controlled All deaths during the investigation period were registered by the Swedish Central Bureau of Statistics To determine the causes of death all death certificates police hospital and autopsy records were checked Sudden death was defined as death occurring within 2 hours of onset of the final symptoms

Survival rates for subgroups of patients were calculated by a life table technique (1) The relative risks of dying were calculated by a comparison of the observed risks during the different follow up intervals with the expected risks in a Swedish population of corresponding age

To create risk profiles and illustrate the interaction effects between different factors AID (Automatic Interaction Detector) analysis was performed (16) The AID analysis may be described as a stepwise regression pro-

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Abbreviations CCU coronary care unit MI myocardial infarction AMI acute MI LBBB left bundle branch block AID Automatic Interaction Detector IHD ischaemic heart disease

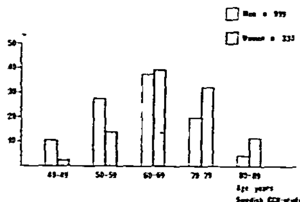


Fig 1 Age and sex distribution of patients with AMI discharged alive from hospital

gram where one variable e g death is considered dependent The variables may be qualitative or quantitative A non-symmetrical branching process is employed to subdivide the sample into series of subgroups which maximizes the ability to predict values of the dependent variable

For testing the significance of differences of proportions the χ^2-test was used Degrees of significance were tested at the 5 1 and 0 1% level

Definitions

Previous myocardial infarction (MI)—a history of MI verified from a hospital record Previous heart failure—a story of digitalis therapy or diuretic therapy not given hypertension Left heart failure—presence of either

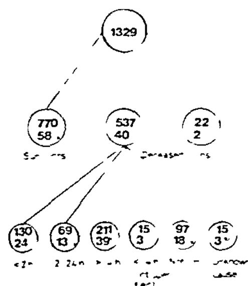


Fig 2 Results of the five year follow-up The time between onset of final symptoms and death is indicated for patients who died from IHD

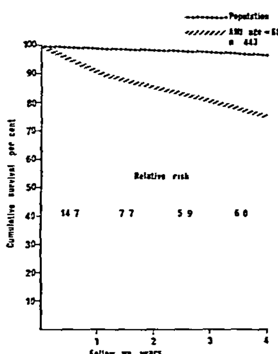


Fig 3 Survival rates and relative risks of death am patients younger than 60 years

basal pulmonary rales a third heart sound or pulmon vascular engorgement on X ray Ventricular tachycar —three or more ventricular ectopic beats in successio

RESULTS

Mortality and prognostic factors

Fig 2 presents the results of the follow-up A to of 537 patients (40%) died Forty per cent of t deaths occurred within 24 hours of onset of the fi symptoms In 18% of the deceased patients t immediate cause of death was not ischaemic he disease (IHD) and in 3% the cause of death co not be identified with reasonable certainty Autop was performed in 299 patients (56%)

Single factors significantly associated with worse long term prognosis are age previous M angina pectoris heart failure hypertension a diabetes as well as the following findings during t CCU period left heart failure supraventricul tachycardia atrial fibrillation left bundle bran block (LBBB) ventricular tachycardia and SGC maximum The survival rates and relative risks death in patients below 60 years are presented Fig 3 The marked dominance of men in the youn est age group should be remembered (Fig 1)

The factor profiles of patient groups with as e

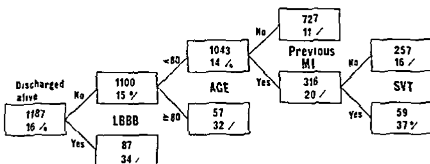


Fig 4 One year risk profiles according to AID analysis The number of patients and the mortality rates are indicated in each prognostic group

treme high and low mortality rates as identifiable by AID analysis during the first year and the first three years respectively are seen in Figs 4 and 5 These analyses concerned a subgroup of 1 187 patients with complete data as regards all single factors of significant prognostic importance Different combinations of age previous MI history or acute signs of left heart failure and LBBB registered some time during the CCU period were found to be of highest prognostic value

Sudden deaths

Only three single factors were significantly associated with sudden death age below 60 years and ventricular and supraventricular tachycardia during the acute phase of the AMI

Fig 6 shows the inverse association between age and the proportion of sudden deaths 37% of the deaths in the youngest age group were sudden compared to only 17% of those in the oldest age group Multivariate analysis including all patients who died within two and four years respectively resulted mainly in the reappearance of ventricular and supraventricular tachycardia as factors associated with sudden death (Figs 7 and 8) During the two periods 25–24% of all deaths were sudden It was only in very small subgroups that tendencies to higher proportions of sudden deaths could be found

Deaths within the first half year of the AMI

The mortality rate during the first six months was 10% which means that 134 (25%) of the 537 deaths during the whole five year follow up occurred during this period Of the early deaths 33 were women with a mean age of 72 years and 101 men mean age 66 years Twenty seven (20%) of the deceased were below 60 years of age and all were men Table I shows the distribution of the early deaths by time interval and mode of death Twenty two per cent of the deaths were sudden but death more than 24 hours after the onset of symptoms was the dominant mode of death

Table II gives the prevalences of previous diseases known to be of prognostic interest as well as

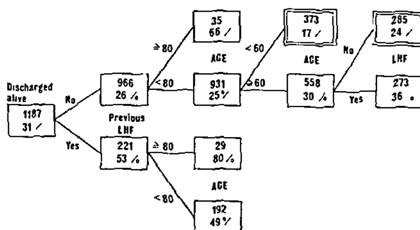


Fig 5 Three year risk profiles according to AID analysis The number of patients and the mortality rates are indicated in each prognostic group

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symptoms registered as sudden On the other hand in young patients with less symptoms aggravation may be less expected and thus more sudden

Apart from low age only ventricular and supraventricular tachycardia registered in the CCU were associated with an increased risk of sudden death The multivariate analyses confirmed these findings but they also showed that it is difficult to use routine data from the CCUs for estimation of risk of sudden death It should be remembered that the arrhythmias associated with sudden death were also included among the factors associated with a generally poor prognosis Serious ventricular arrhythmias during the late part of the hospital period after AMI has previously been shown to be associated with sudden death (9) Thus repeated evaluation of the patients tendencies to ectopic ventricular activity seems to be of additional prognostic value The present finding of supraventricular tachycardia as related both to sudden death and to a worse long term prognosis is in accordance with other studies where signs of heart failure such as cardiac enlargement proved as indicative of an increased risk of sudden death as serious ventricular arrhythmias registered before discharge (14)

Trials of secondary prevention with β receptor g agents in survivors of AMI indicate a reduction of sudden deaths and reinfarctions during the first years after the AMI (12 18) The results of such treatment in patients above 65 years of age are not known but the present findings may indicate that the gain as regards a decrease of the incidence of sudden death is mainly to be expected in younger age groups

Early deaths after discharge

Mortality among immediate survivors of MI is known to be high especially during the first year (4) For secondary prevention this is of great practical importance and considerable efforts have been made to identify not only groups with an increased risk of sudden death but also those prone to death within a few months after discharge Moss et al (11) tried to characterize patients with high risks of death in the first four months after an AMI In 518 such patients (≤65 years of age) four month mortality rates varied from 3 to 14% Patients of the high risk group 16% of all patients had at least two of the following characteristics 1) Previous angina pectoris at ordinary levels of activity or at rest 2) Hypotension or congestive heart failure in the CCU 3) ≥20 Ventricular ectopic beats per hour on a six hour ECG tape recording made before discharge

In the present study 134 patients discharged alive died within the first six months after the AMI The prevalence of factors well known to be associated with a less favourable long term prognosis proved to be significantly increased in this patient group as compared to those who lived for a longer period As compared to patients who died suddenly, however there were few differing characteristics So patients with short survival time showed a highly significant accumulation of bad prognostic signs already during the acute stage of their AMI The majority of them had one or two of the following risk factors previous MI left failure or serious arrhythmias or conduction disturbances registered in the CCU The most frequent and important factors were heart failure in the CCU and a history of MI These findings are in agreement with those of Moss et al (11) and indicate the problem of secondary prevention with β receptor blocking agents in high risk patients with more or less severe heart failure after AMI

Another challenge is the elusive clinical profile of those 17% of the early deceased who had shown none at all of the above bad prognostic signs (Table III) Investigation for new and more specific secondary risk factors is in constant demand Most of our present important prognostic factors regarding survivors of AMI were already well known in the 1930s (10)

ACKNOWLEDGEMENT

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REFERENCES

1 Chiang C L Introduction to stochastic processes in biostatistics Wiley New York 1968
2 Cole D R Singian E A & Kate L N The long term prognosis following myocardial infarction and some factors which affect it Circulation 9 321 1954
3 Coronary Drug Project Research Group Factors influencing long term prognosis after recovery from myocardial infarction—three year findings in the Coronary Drug Project J Chronic Dis 27 267 1974
4 Frank C W The course of coronary heart disease Factors relating to prognosis Bull NY Acad Med 44 950 1968

5 Geismar P Iversen E Mosbech J & Deyer K Long term survival after myocardial infarction A national follow up study on 647 patients in Denmark Int J Epidemiol 2 257 1973
6 Helmers C Short and long term prognostic indices in acute myocardial infarction Acta Med Scand (Suppl) 555 1973
7 — Assessment of 3 year prognosis in survivors of myocardial infarction Br Heart J 37 593 1975
8 Henning R & Lundman T Swedish co-operative CCU study Acta Med Scand (Suppl) 586 1975
9 Kotler M N Tabatznik R Mower M M & Tominaga S Prognostic significance of ventricular ectopic beats with respect to sudden death in the late postinfarction period Circulation 47 959 1973
10 Master A M Dack S & Jaffe H L Coronary thrombosis An investigation of heart failure and other factors in its course and prognosis Am Heart J 13 330 1937
11 Moss A J DeCamilla J Davis H & Bayer L The early posthospital phase of myocardial infarction Circulation 54 58 1976
12 Multicentre International Study Improvement in *prognosis of myocardial infarction by long term* beta adrenoreceptor blockade using practolol Br Med J 3 735 1975
13 Norris R M Caughey D E Deeming L W Mercer C J & Scott P J Coronary prognostic index for predicting survival after recovery from acute myocardial infarction Lancet 2 485 1970
14 Rehnqvist N Prognostic weight and natural history of ventricular arrhythmias after an acute myocardial infarction Eur J Cardiol In press 1978
15 Romo M Factors related to sudden death in acute ischaemic heart disease A community study in Helsinki Acta Med Scand (Suppl) 547 1973
16 Sonqvist J A & Morgan J N The detection of interaction effects Survey Research Center Monograph No 35 Institute for Social Research The University of Michigan Ann Arbor Michigan 1964
17 Vedin A Wilhelmsen L Wedel H Pettersson B Wilhelmsson C Elmfeldt D & Tibblin G Prediction of cardiovascular deaths and non fatal reinfarctions after myocardial infarction Acta Med Scand 201 309 1977
18 Vedin J A Wilhelmsson C & Werko L Chronic alprenolol treatment of patients with acute myocardial infarction after discharge from hospital Effects on mortality and morbidity Acta Med Scand (Suppl) *575 1975*

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1 Chiang C L Introduction to stochastic processes in biostatistics Wiley New York 1968
2 Cole D R Singian E A & Kate L N The long term prognosis following myocardial infarction and some factors which affect it Circulation 9 321 1954
3 Coronary Drug Project Research Group Factors influencing long term prognosis after recovery from myocardial infarction—three year findings in the Coronary Drug Project J Chronic Dis 27 267 1974
4 Frank C W The course of coronary heart disease Factors relating to prognosis Bull NY Acad Med 44 950 1968

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Cardiac Rupture in Acute Myocardial Infarction

A Review of 72 Consecutive Cases

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ABSTRACT The occurrence of rupture of the external cardiac wall (CR) in a consecutive series of 2 244 admissions with confirmed acute myocardial infarction (AMI) has been analysed The series comprises the unselected admissions to a single department, evaluated according to uniform criteria, with post mortem examination in 95% of fatal cases The incidence of CR was 3 2% af all cases of AMI, and 12 6% of all deaths CR was significantly more frequent in women than in men and in both sexes significantly more frequent after the age of 60, though the age distribution did not differ significantly from that of the patients dying from other causes Moreover, CR was significantly more frequent in anterior wall infarctions, and in patients with no previous AMI The majority of CR (84%) occurred within the first week, and a considerable part (32%) within the first 24 hours after the onset of infarction According to findings at autopsy, CR was accompanied particularly often (in 71%) by complete occlusion of a major coronary artery Autopsy findings gave no evidence that attempts at resuscitation, including external cardiac massage (65 patients), as well as intracardial injections (55 patients) and transthoracic introduction of a pace catheter (3 patients) could have any connection with the development of CR The usual strict immobilization of the patients was applied during the first part of the series but in the latter half (1 337 admissions) the patients were [a]llowed to be out of bed from the first day with no [i]mposed immobilization at all The incidence of CR [d]id not change significantly throughout the period the indication for maintaining the traditional [immobil]ization of patients with AMI is questioned

[K]ey words cardiac rupture acute myocardial infarction [p]hysical activity
[A]cta Med Scand 205 11 1979

[M]yocardial rupture (CR) is a well known and almost invariably fatal complication to acute myocardial infarction (AMI) (1) Physical activity in [p]atients with fresh infarction has been suspected as a precipitating factor and this assumption has been the major reason for the traditional prescription to these patients of a period of strict bed rest followed by gradual mobilization and gradual return to work

In this paper the incidence of rupture of the free wall and the hospital lethality have been followed in a large consecutive series of patients with AMI treated with and without strict bed rest and immobilization during the acute phase

PATIENTS AND METHODS

The report is based on a consecutive series of patients with AMI admitted to a medical department during the years 1959–73 and from 1966 to the Coronary Care Unit (CCU) of Copenhagen County Hospital Glostrup The hospital serves a well defined area from which all cases of suspected AMI care are admitted without any preselection in relation to age or complicating diseases This 14-year period can be divided into four stages representing development from the non monitored general medical ward with the traditional coronary regime to the full time monitored CCU without immobilization of the patients

Period A From 1959 to 1966 a general medical ward without ECG monitoring All patients with AMI were prescribed strict bed rest for up to eight days followed by gradual mobilization and discharge after four weeks

Period B In the following two years patients with AMI were monitored in a CCU for an initial period averaging three days and were then transferred to a general medical ward During their stay in the CCU they had some liberty to move around but after transfer to the medical ward the traditional regime of immobilization was followed

Period C+D During the subsequent four years the CCU was enlarged so that patients with confirmed AMI were now kept for the whole period of admission i e for surviving patients at least 21 days after AMI From 1968 to 1970 (period C) the patients were monitored for an average of five days From 1971 to 1973 (period D) and later the

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Abbreviations CR = myocardial rupture AMI = acute myocardial infarction CCU = coronary care unit

Table I *Incidence of CR and distribution by sex and age in ?744 patients with AMI*

	Males				Females				Both sexes			
	All cases of AMI		CR in AMI		All cases of AMI		CR in AMI		All cases of AMI		CR in AMI	
Age group	*n*	%	*n*	% of all AMI	*n*	%	*n*	% of all AMI	*n*	%	*n*	% of all AMI
<40	39	?4	0		5	0 8	0	–	44	1 9	0	
40–49	235	14 4	1	0 4	34	5 5	0		?69	11 9	1	0 4
50–59	499	30 7	9	1 8	96	15 6	?	? 1	595	6 5	11	1 9
60–69	473	?9 1	13	? 8	183	?9 6	10	5 5	656	?9 ?	23	3 5
70–79	?95	18 1	15	5 1	2?5	36 5	1?	5 3	5?0	23 2	?7	5 ?
≥80	86	5 3	4	4 ?	?4	11 9	6	8 1	160	? 1	10	6 3
Total	1 62?	100	4?	? 6	61?	100	30	4 9	? ?44	100	7?	3 ?

patients were continuously ECG monitored for the whole of the stay in the latter part by telemetry In this four year period no prescribed immobilization was imposed on the patients as in most other diseases bed rest was applied only to those patients whose clinical condition required it All other patients were out of bed from the first day dressed and walking freely about during the first days confined to the room by the ? m long cable from the chest electrodes to the ECG amplifier on the wall but still able to sit at the table for meals and go to the wash-basin for personal toilet During the rest of the hospital stay the patient could walk all over the department carrying in a ?l telemetry transmitter from which the ECG was sent continuously to the observation room A daily quantitation of the physical activity expended by each patient was not attempted systematically

The present series comprises all patients treated in the CCU during the periods in question and meeting the criteria of WHO (30) for the diagnosis AMI excluding only those who arrived unconscious after an out-of hospital cardiac arrest Clinical data according to uniform definitions were available for all patients from a prospectively planned data base

Post-mortem examination was available in 95 1% of the fatal cases The diagnosis of CR was never made on a clinical basis but always on the autopsy finding of significant amounts of blood in the pericardium together with either a regular perforation of the free myocardial wall or a dissecting infiltration of blood in the myocardium Thus the definition of CR and the present series of patients do not comprise cases of *rupture of the* septum or a papillary muscle or other inner structures For all patients presenting a CR at autopsy clinical data were re-evaluated in retrospect

Statistical analysis was performed by either the Kruskall-Wallis test or the χ^2-test with Yates correction A level of significance at 5% was chosen

RESULTS

Among ??44 patients with confirmed AMI 571 (25 4%) died during the stay in hospital Autopsy was performed in 543 of the 571 deaths demonstrating a CR as cause of death in ?? Thus the overall incidence of confirmed CR was 3 ?% of all patients and 1? 6% of all the fatal cases

Table I shows the sex and age distribution (decades) of the patients with CR and of all patients with AMI The incidence of CR was significantly higher in the age group over 60 years than in the younger age groups (4 5% against 1 3% $p<0.0005$) and significantly higher in women than in men (4 9% against ? 6% $p<0.001$) The mean age of the 4? men was 68 5 years (range 46–90) and of the ?0

Table II *Cases of CR compared with all considered cases of AMI as well as fatal cases in four consecutive periods*

Period A non-monitored bed ward strict immobilization (7 y) period B short-term CCU partial immobilization (? y) period C–D full-term CCU no immobilization (?+? y)

	Period			
	A	B	C	D
AMI (total no)	422	485	55?	785
No of deaths from AMI	151	118	140	16?
No of autopsied AMI cases	14?	114	13?	155
No of CR demonstrated at autopsy	8	16	?0	28
CR in AMI (%)	1 9	3 3	3 6	3 6
Statistical significance*		n s	n s	n s
Lethality in AMI (excl ruptures) (%)	33 9	?1 0	1? 7	17 1
Statistical significance*		$p<0.0005$	n s	$p<0.05$
CR in deaths from all causes (%)	5 3	13 6	14 3	17 3
Statistical significance*		$p<0.05$	n s	n s

* χ^2 test with Yates correction n s not significant

Table III *Interval from onset of symptoms to occurrence of CR in 68 patients*

Days	No of pats Total	♂	♀
<1	22	15	7
1–2	8	5	3
2–3	9	7	2
3–4	5	4	1
4–5	7	2	5
5–6	6	2	4
7–8	4	2	2
8–9	1	–	1
12–18	4	3	1
19–24	2	2	–
Unknown	4	–	4
Total	72	42	30

women 72 5 years (range 56–85) This difference is not statistically significant It will be seen that within each ten year group a higher incidence of rupture was found for women than for men Patients dying with CR were significantly older than those who survived the infarction but their age distribution did not differ significantly from that of the patients who died from other causes during their acute infarction

Table II shows the incidence of CR compared with all considered cases and fatal cases in the various periods The difference in overall incidence between periods A and B was not significant (0 30 $<p<0$ 20) Among the fatal cases the share of CR did increase significantly from period A to period B ($p<0$ 05) but the overall lethality in the same period decreased considerably

The lapse of time from onset of symptoms to time of rupture could be established in 68 patients As appears from Table III CR occurred during the first 24 hours in 22 patients (32%) and within the first week in 57 (84%) Concerning the median time lapse from admission to CR no difference was found between the three periods of strict bed rest (period A) partial mobilization (period B) and no immobilization (periods C and D) Neither was there a significant difference between the three periods with regard to the median time from admission to death from CR and to death from other causes (Table IV)

Concerning the physical activity performed prior to death from CR the study was confined to the 64 patients from periods B C and D in which no immobilization was prescribed Of these 64 patients 20 who died during the first 24 hours and 16 who died later had been kept in bed from admission until the cardiac rupture Another 6 patients were known to have performed some physical activity immediately prior to the terminal event such as toilet visit using a commode or sitting dressed in the room having a meal For the remaining 22 patients no reliable information was available about physical activity prior to the terminal event

Previous AMI was registered in 11% of all cases with CR and in 23% of all other cases This difference is significant ($p<0$ 05)

Regarding the location of the infarct according to ECG there were significantly more anterior infarctions in the group with CR than in the group of deaths from other causes ($p<0$ 05) (Table V)

A penetrating defect was demonstrated at autopsy in the myocardium of 60 patients Apart from

Table IV *Lethality from CR compared with all other deaths and interval from admission to death among patients with AMI in three consecutive periods with different policy of immobilization*

	Period A (n=422)		Period B (n=485)		Period C+D (n=1 337)	
	Deaths from CR	Deats from other causes	Deaths from CR	Deaths from other causes	Deaths from CR	Deaths from other causes
No of pats	8	120	16	89	48	235
Days from admission to death						
1	3	57	5	29	19	97
2– 5	3	26	8	30	22	62
6–10	0	19	1	9	4	25
11–21	2	18	2	21	3	51
Median	3 3	3 4	3 5	4 1	2 9	3 3

increase significantly in the transition from a period of traditional immobilization to the present arrangement with no immobilization at all. This experience seems to confirm the impression that the common routine of strict immobilization of AMI patients for fear of provoking CR must be regarded as unfounded and superfluous.

We may quote the view of Rose (24) that "The evidence linking ventricular rupture with early mobilization is thus unclear ... the onus of proof will be on physicians who would restrict their patients' lives to show evidence that their interference is beneficial".

REFERENCES

1 Bates R J, Beutler S, Resnekov L & Anagnostopoulos C E. Cardiac rupture—challenge in diagnosis and management. Am J Cardiol 40: 429, 1977.
2 Becker A E & van Mantgem J-P. Cardiac tamponade. A study of 50 hearts. Eur J Cardiol 3: 349, 1975.
3 Bøe I G, Mogensen L, Nyquist O, Orinius E & Sjögren A. Studies of myocardial rupture with cardiac tamponade in acute myocardial infarction. Chest 61: 4, 1972.
4 Christensen J B & Pedersen O L. Cardiac rupture after acute myocardial infarction. Dan Med Bull 23: 207, 1976.
5 Cobbs B W, Hatcher C R & Robinson P H. Cardiac rupture. Three operations with two long-term survivals. JAMA 223: 532, 1973.
6 Coe W S. Cardiac work and the chair treatment of acute coronary thrombosis. Ann Intern Med 40: 4, 1954.
7 Friedman H S, Kuhn L A & Katz A M. Clinical and electrocardiographic features of cardiac rupture following acute myocardial infarction. Am J Med 50: 709, 1971.
8 Gjøl N. Cardiac rupture and acute myocardial infarction. Geriatrics 32: 126, 1977.
9 Griffith G C, Hegde B & Oblath R W. Factors in myocardial rupture. Am J Cardiol 8: 792, 1961.
10 Haskell W K. Physical activity after myocardial infarction. Am J Cardiol 33: 776, 1974.
11 Hatle Ö. Cardiac rupture in recent myocardial infarction. Acta Pathol Microbiol Scand (A) 51: 501, 1977.
12 Helmers C. Short and long-term prognosis indices in acute myocardial infarction. Acta Med Scand (Suppl) 555, 1974.
13 Hofvendahl S. Influence of treatment in a coronary care unit on prognosis in acute myocardial infarction. Acta Med Scand (Suppl) 519: 1, 1971.
14 Jetter W W & White P D. Rupture of the heart in patients in mental institutions. Ann Intern Med 21: 783, 1944.
15 Kaveman D A. Myocardial rupture. A study in non-psychotic and psychotic patients. Can Med Ass J 82: 1105, 1960.
16 Lautsch E V & Lanks K W. Pathogenesis of cardiac rupture. Arch Pathol 84: 264, 196
17 Lewis A J, Burchell H B & Titus J L. Clinical and pathologic features of postinfarction rupture. Am J Cardiol 23: 4, 1969.
18 London R E & London S B. Rupture of the heart. Circulation 1: 307, 1965.
19 Maher J F, Mallory G K & Laurenz G A. Rupture of the heart after myocardial infarction. N Engl J Med 225: 1, 1956.
20 van Mantgem J P & Becker A E. Development of cardiac rupture as initial sign of acute myocardial infarction. Br Heart J 38: 1077, 1976.
21 Meurs A A H, Vos A K, Verbeek J B & Gerbrandy J. Electrocardiogram during cardiac rupture by myocardial infarction. Br Heart J 32: 232, 1970.
22 Naeim F, de la Maza L M & Robbins S L. Cardiac rupture during myocardial infarction. Circulation 45: 1231, 1972.
23 O'Rourke M F. Subacute heart rupture following myocardial infarction. Lancet 2: 124, 1973.
24 Rose G. Early mobilization and discharge after myocardial infarction. Mod Concepts Cardiovasc Dis 41: 59, 1972.
25 Sievers J. Cardiac rupture in acute myocardial infarction. Geriatrics 21: 125, 1966.
26 Sigler L H. Sudden death due to cardiac rupture in myocardial ischaemia and infarction. NY State J Med 69: 794, 1969.
27 van Tassel R A & Edwards J E. Rupture of the heart complicating myocardial infarction. Chest 61: 104, 1972.
28 Thompson P L, Jenzer H R, Lown B & Lohrbauer L A. Exercise during acute myocardial infarction: an experimental study. Cardiovasc Res 6: 42, 1973.
29 Wessler S, Zoll P M & Schlesinger M J. The pathogenesis of spontaneous cardiac rupture. Circulation 6: 334, 1952.
30 WHO Regional Office for Europe. Ischaemic Heart Disease Registers. Report of the Fourth Working Group. Copenhagen 1970.

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Detection of Asystole, Ventricular Fibrillation and Ventricular Tachycardia with Automated ECG Monitoring

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ABSTRACT The performance of an automated arrhythmia monitoring system has been studied with reference to asystole, ventricular fibrillation (VF) and ventricular tachycardia (VT) during two periods of routine monitoring The system distinguished normal supraventricular and ventricular ectopic beats with the use of rhythm and QRST data VF was recognized from the power spectrum of the ECG A total of 17 arrhythmia diagnoses (alarms) could be made by the system A write-out, with a delayed ECG and a diagnostic message in parallel was recorded at each alarm In the first part of the study a continuous ECG used as a reference, was recorded from all patients in the coronary care unit during a 4-week period The number of manually recorded episodes of asystole VF and VT was 24 4 and 44 respectively Out of these 92 100 and 82% respectively, were reproduced on the alarm write-outs A correct diagnosis was made by the automated system in 33 75 and 70% of true asystole VF and VT events respectively Three false asystole alarms all due to loose electrodes were reported during this part of the study In the second part of the study all arrhythmia write-outs were analysed for six months for the occurrence of VF and VT alarms Out of 22 episodes of VF 73% were reported as VF and the remaining as VT Of 534 VT episodes 82% were diagnosed correctly The proportions of correct false positive VF and VT detections were approximately 1 5 and 4 1 Compared with conventional monitoring the automated system seems to specifically improve the detection rate of VT

Key words Arrhythmias computer coronary care unit

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Arrhythmia monitoring forms the basis of the coronary care unit (CCU) The accuracy of ECG monitoring depends on many factors such as the presentation and storage of ECG signals the skill and time available for the human observer and the performance of any automated monitoring device added to the system In routine clinical monitoring a substantial proportion of the arrhythmias is never detected and great efforts have been made to improve this with the use of automated arrhythmia monitoring systems Such a system has been developed and installed in the CCU of Sodersjukhuset Stockholm The performance of the system was evaluated recently during 1 000 h of monitoring in 55 patients (3) However in respect of some serious arrhythmias this could not be done owing to a lack of such episodes

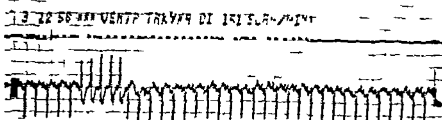
In the present study which consists of two parts the performance of the automated monitoring system in asystole ventricular fibrillation (VF) and ventricular tachycardia (VT) has been evaluated during long term routine use In the first part the number of detected and undetected events was estimated during 4 weeks using a continuous ECG recording as reference In the second part true and false VF and VT alarms were studied for six months by analysis of computer generated alarm write outs

PATIENTS

All patients admitted to the CCU with suspected heart disease or a rhythm disorder were included in the study Each admission was treated as a different patient Acute myocardial infarction (AMI) was diagnosed from a typical history and accepted enzyme and/or ECG criteria The

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Abbreviations CCU=coronary care unit VF=ventricular fibrillation VT=ventricular tachycardia AMI=acute myocardial infarction VB=ventricular ectopic beat SVT=supraventricular tachycardia SVB=supraventricular ectopic beat HR=heart rate BBB=bundle branch block



Fg 1 Sample alarm write out in VT Upper tracing shows room number time priority level (xxx) arrhythmia d agnos s and HR

performance of the system was evaluated during two non-overlapping periods lasting 4 weeks (65 pats) and 6 months (440 pats) The total monitoring time was about 3400 and 21000 h respectively AMI was diagnosed in about 40% of the patients

METHODS

Principles f r a to nate l arrhythm ia analysis

The principles for arrhythmia analys s of the monitoring system have been described briefly elsewhere (2) No essential changes in the computer algorithms have been made s nce the earl er study The mon toring system wh ch analyses the b polar ECG from up to eight patients s multaneously has been des gned to recognize a number of arrhythmias and artefacts These events result in alarms grouped into three priority levels

The highest priority level comprised asystole VF and VT Asystole was diagnosed when no ECG complexes were detected for 5 sec in a noise-free s gnal VF was recognized by the computer from the power spectrum of the ECG after 5 sec of abnormal rhythm VT was diagnosed when *four or more* consecutive ventricular ectop c beats (VBs) with a frequency above 1?0 had been detected by the system VT could also be recogn zed from the power spectrum of the ECG In this analys s how ever VT with a frequency above ?40 was class fed as VF The alarms in the second priority level included two three or more than three consecutive VBs extreme bradycardia (heart rate (HR) less than 35/min) VB of R on T type id oventricular rhythm ventricular b geminy and su*praventricular tachycard a (SVT) An SVT alarm re* qu red four or more consecutive supraventricular ectop c beats (SVBs) with a frequency above 1 0 The lowest priority alarms cons sted of multiform VBs miss ng QRS (at least 50% prolongation of regular RR intervals) more than five VBs/SVBs per min and tachy /bradycardia The rate l mits for the last two alarms could be chosen manu ally but were set automat cally to 1?0 and 50 at the onset of monitoring

The system also produced a *number of alarms referring* to the conf guration of the ECG s gnal Three of these were assigned to the second priority level namely check electrodes new QRS and what has happened? At *any of these* alarms as well as at any arrhythmia alarm a ?-channel M ngograph was activated by the computer The room number of the patient the time of the alarm and the computer diagnos s or remark were printed on one channel (Fg 1) HR was also recorded in tachycard a or bradycard a alarms On the other channel a 5 sec retroact ve ECG was d splayed at a paper speed of 10 mm/sec Th s ECG had been f ltered in order to reduce artefacts Usually the write-out had a durat on of 15 sec All alarm cond t ons were ranked even with n the same pri ority level and the h ghest priority cond tion was always *selected by the computer* when a part cular ECG event fulf lled the criteria for more than one diagnos s Repea ed arrhythmias of the same type were not presented on the write-outs if they occurred within the reset time H ghest priority alarms could be retriggered *after 10 sec* Reset time for the second and th rd priority alarms was 4 min

The pat ent electrodes (Stemmen mon toring electrodes) were rout nely placed over the sternum but sometimes this placement resulted in a less satisfa tory conf guration of the ECG as indicated by one of the fol lowing messages "unfavourable placement of electrodes or low ampl tude" These messages were d played on video screens in the central stat on and in the patient *rooms as long as the cond tion pers sted During this time* the computer analysis was inh b ted for all but the HR alarms and the h ghest priority arrhythm a cond ti ns The mon toring system and all alarms are described n detail elsewhere (6)

Pr nciples f r ECG class f cation by the pl ysician

The ph s cian s interpretation of arrhythmias was made off-l ne from s ngle-channel paper record ngs at a speed of 10 mm/sec

The def n tion of VBs and SVBs in the manual cla s f*cation of heart beats was based on prematurity* QRST aberrancy and QRS width In cases with narrow QRS complexes (QRS t me <0 11 sec) a def n te QRS prolonga t on was requ red for a VB diagnos s Premature and aberrant beats wh ch were ne ther narrow nor widened were usually classif ed as SVBs When poss ble the P wa e was also taken into account and runs of widened beats with abnormal morphology and un hanged PQ interval (n term ttent bundle branch block (BBB)) were class f ed a *normal The initial part of the QRS complex* and the distribution of RR intervals were also cons dered n order to d stinguish aberrantly conducted beats from true VBs Thus d stinction may be mpossible particularly in rap d irregular rhythm and therefore in cases of great uncertainty in the manual interpretation abnormal beats were classif ed as SVBs The case records were stud ed n a number of cases and such information improved the

Table I *Detection of asystole by the automated monitoring system during 4 weeks of routine monitoring*

Arrhythmia diagnosis in alarm write-out	No of episodes
Asystole	8
Extreme bradycardia	6
Bradycardia	1
What has happened?	5
Check electrodes	2
Undetected	2
Total	24

diagnostic accuracy in patients who had VT on admission or the WPW syndrome and in patients in whom an oesophageal lead recording was available

In the manual interpretation the same definitions of VT and SVT were used as in the automated classification Asystole was diagnosed when the RR interval exceeded 5 sec VF was defined as rapid oscillations with varying amplitudes and a duration of 5 sec or more and a QRS morphology different from the ordinary complexes

In the analysis of the alarm write outs only the first complete arrhythmia print-out of the computer was taken into account i e if the first complexes of a true VT episode were classified as paired VBs but the alpha numeric print-out was interrupted by a VT alarm the former incomplete statement was neglected In the terminal phase or during resuscitation only the first asystole or VF or VT was analysed

During the 6-month period one patient with AMI who had more than 1 000 episodes of SVT with marked aberra tion was excluded from the results In this patient as well as in a few others with atrial fibrillation or frequent true VT the VB detection function of the automated system was deselected in order to reduce the number of alarms During the 4-week study this was done for two patients One of them had atrial fibrillation and a great number of abnormal beats due to digitalis intoxication manual clas sification was impossible and this patient was excluded from the study The other patient exhibited numerous true VT alarms and VB detection was suspended about 1 h after admission

During the 6-month period the computer analysis was interrupted on one occasion for 26 hours due to overheat ing of the computer as a result of unsufficient ventilation Interruptions of the computer write outs for other reasons such as changing the ink bottle or paper feed were considered negligible

The nurses opinion of the automated system was studied during the 6-month period in an anonymous in quiry which will be reported briefly in the discussion

Evaluation of alarms in true asystole VF or VT

In the first part of the study which lasted for 4 weeks the automated arrhythmia monitoring system was evaluated by comparing true arrhythmia prevalence regarding asystole VF and VT with the computer-generated alarm write-outs A continuous bipolar ECG was recorded with a paper speed of 10 mm/sec on an 8-channel Mingograph (Elema 61) and these recordings were analysed manually beat by beat without knowledge of the classification of the computer The alarm write-outs were not studied until the collection and interpretation of the continuous ECG rec ords had been completed Only the first 10 episodes of asystole or VF or VT in each patient were studied A maximum of seven patients could be recorded simultane ously since one channel of the recorder was reserved for a computer generated alphanumeric time marking This channel also indicated the onset or termination of auto mated monitoring for a particular patient as well as the activation of a conventional HR-dependent alarm system which operated in parallel with the automated monitoring system During this part of the study false positive asystole alarms were also counted

Evaluation of alarms reported as VF or VT

During the 6-month period the ratio of true to false alarms was obtained for VF and VT by studying both channels of the alarm write-outs The reason for false alarms was analysed whenever detected If the arrhythmia episode studied first in a computer write-out was detected correct ly any second episode on the same write-out within the reset time was disregarded A continuous arrhythmia episode could only result in one correct alarm and any false alarms following the correct one were neglected

Performance of the system in VT was compared in AMI and non AMI patients

RESULTS

Accuracy of alarms in asystole VF and VT

The prevalence of asystole VF and VT during a 4 week period was estimated from a continuous ECG paper recording

Asystole A total of 24 asystole episodes were seen in five patients Analysis of alarm write outs revealed 21 asystole periods (isoelectric ECG/RR >5 0 sec) in the write-outs (Table I) In one case of true asystole only 4 0 sec of isoelectric ECG had been captured on the write out The computer re ported this event as bradycardia Eight correct asystole alarms were given In six cases of asystole extreme bradycardia was reported and this alarm was usually triggered a few seconds before the true asystole event due to a gradual slowing of the HR Asystole alarms were blocked at a certain total energy of the signal Therefore correct detection of asystole was inhibited in some cases by baseline shifts or marked and persistent P waves in total

Table II *Performance of the automated monitoring system in VF and VT during 4 weeks of routine monitoring*

True event	Classification of the computer	No of episodes
VF	VF	3
	VT	1
Total no of true episodes of VF		4
VT	VT	31
	VF	1
	Consecutive VBs	3
	Tachycardia	1
	Undetected	8
	Preceded by artefacts	2
	Unfavourable placement of electrodes	2
	Multifocal rhythm	1
	Unknown	3
Total no of true episodes of VT		44

block This explained the absence of asystole alarms in the write outs that were labelled "extreme bradycardia" or "what has happened?" Maximal delay from last QRS to the alarm in the above situations was 15 sec In two cases artefacts inhibited the asystole alarm and "check electrodes" was reported after 40 and 60 sec respectively On two occasions asystole occurred but did not lead to an alarm write out by the system In these patients a *slow multifocal idioventricular rhythm* was observed for some minutes before the asystole period and alarms for VF and extreme bradycardia had been given prior to the asystole events

Nine false positive asystole alarms were seen during the period six of which occurred when the bedside monitor was disconnected without a previous stand by operation Three false asystole alarms resulted from a loss of signal due to electrode problems These alarms which had been preceded by the message "check electrodes" followed short lasting artefacts simulating true ECG complexes that occurred after more than 30 sec of isoelectric ECG

Ventricular fibrillation occurred four times in four patients The computer diagnosis was VF in three and VT in one patient (Table II) Delay from onset of VF to the generation of the alarm varied between 5 and 14 sec

Ventricular tachycardia A total of 44 episodes were seen in 19 patients The results of the computer classification are shown in Table II Thirty-one correct VT detections were recorded One short VT was immediately preceded by an artefact that gave rise to a false VF alarm This alarm inhibited the presentation of a VT alarm and is reported as VF in Table III One true VT following a period of idioventricular rhythm was classified as "tachycardia" All detected VTs whether diagnosed correctly or not resulted in an alarm within 5 sec from the onset of the arrhythmia In five instances VT was unrecognized due to the presence of artefacts unfavourable ECG configuration of ordinary complexes or multifocality In one case of multifocal VT no identical VBs followed one another Such a criterion had been set up for a VT diagnosis by the computer On three occasions VT was undetected for unknown reasons One of these arrhythmias was possibly missed due to a low amplitude another was short (4 beats) and preceded within 4 min (reset time) by correct alarms for VBs in succession The latter arrhythmia would not have been reproduced on the alarm write out even if detected and alarmed as consecutive VBs

Two VT episodes correctly detected by the automated system occurred when the continuous ECG recording was interrupted Also one true VT was detected by the computer but was not notified in the manual interpretation These three VT alarms were not included in the results

Accuracy of reported alarms for VF and VT

The computer generated alarm write-outs were analysed in all patients during 6 months for the occurrence of VF or VT alarms

Ventricular fibrillation True VF was seen on 22 occasions in 11 patients (Table III) In six cases this arrhythmia occurred prior to death The computer gave a correct diagnostic message in 16 and a VT alarm in 6 of the 22 episodes of VF False positive VF alarms were relatively common and caused by rapid VT (HR >240/min) in five cases Some patients with rapid atrial fibrillation or flutter combined with left BBB showed relatively high figures for false positive VF detection Artefacts resulting in VF alarms were usually caused by loose electrodes Relatively rare causes of false VF alarms were VBs in succession SVT idioventricular rhythm and unfavourable configuration of the ECG

Table III *Classification of VF and VT by the automated monitoring system in 440 patients during 6 months of routine monitoring as evaluated from the computer generated alarm write outs*

RUN = 2 or more VBs in succession SR = sinus rhythm AF = atrial fibrillation or flutter
Figures within parentheses indicate the number of patients contributing to the preceding figure

Classification of the computer	True condition VF	VT	RUN	SVT	AF + aberrant conduction/ BBB	SR + aberrant conduction/ BBB	Low QRS + high P or T	Artefacts	Other
VF	16 (10)	14 (10)	5 (5)	2 (2)	27 (6)		5 (4)	24 (23)	3 (3)
VT	6 (1)	438 (99)	9 (9)	31 (11)	51 (10)	8 (5)	8 (3)	8 (6)	3 (2)
RUN		64 (31)							
SVT		4 (4)							
Tachycardia		7 (4)							
Other		7 (5)							

complex (high P or T in combination with a low QRS amplitude) The proportion of true false positive VF alarms was 1 5

Ventricular tachycardia VT was recognized by the computer on 534 occasions in 109 patients (Table III) A correct alarm was present in 438 instances (82%) On 14 occasions (excluded from Table III) the write-out contained a VT but it was apparent that this arrhythmia had not activated the automated system The above figures however reflected neither the true number of VT episodes nor the true number of undetected events False negative VT alarms were seen on 96 occasions in 35 patients A majority of these arrhythmias were reported as consecutive VBs and resulted usually from undetected VBs in a short run of VBs and apart from a number of VF alarms in true VT other computer diagnoses in VT were uncommon A total of 118 false positive VT alarms were reported in 38 patients A vast majority resulted from a conduction disturbance or BBB occurring intermittently in conjunction with either SVT atrial fibrillation or atrial flutter Three patients contributed 32 false VT alarms Noisy signal was the cause of false VT alarms on 8 occasions In some false positive VT and VF alarms minor artefacts probably contributed to the false alarm These alarms were registered as runs of VBs SVT or AF in Table III The ratio of true false positive VT alarms was 4 1

Performance of the system in patients with and without AMI

The percentage of correctly detected VTs was lower in AMI than in non-AMI patients during the 6-month period The difference was relatively small 8% and is possibly explained by a higher proportion of short runs of VT in AMI (unpublished observation) The number of false positive VF or VT alarms was slightly higher in patients with than without AMI but was proportional to the total monitoring time in the two patient groups

DISCUSSION

In the present study an automated arrhythmia monitoring system has been evaluated in some serious arrhythmia conditions such as asystole ventricular fibrillation and ventricular tachycardia Such arrhythmias comprised 1–2% of all arrhythmias reported by the system Because of the rarity of the arrhythmias studied a rather long period had to be covered in order to obtain an appropriate number of arrhythmias The 4 week period was not quite long enough for proper evaluation of the VF alarms since only four episodes occurred during this period In these cases a highest priority alarm was reported which indicates that the sensitivity of the system to VF is high

The relatively high number of "extreme bradycardia" and "what has happened?" alarms in true asystole was due either to small baseline shifts or to marked P waves in relation to the QRS amplitude in a few patients with total block The asystole alarm was suppressed when the power of the signal exceeded a relatively low level [illegible] a somewhat higher threshold would have a higher detection rate with little or no

false alarms False positive asystole alarms because of electrode problems should be avoidable with bedside equipment for measuring electrode impedance

The number of false positive VF alarms was high in a few patients with BBB particularly if combined with rapid irregular rhythms Changes in the computer programs to decrease the number of false VF alarms in the present monitoring system have been suggested earlier (5)

About 80% of true VTs were detected by the system during a 4-week period This percentage can probably be increased somewhat by paying more attention to the placement and care of the electrodes Apart from the monitoring status alarms the staff received no particular instructions about the electrodes Previous studies from well equipped CCUs with nurse based ECG monitoring round the clock have shown figures of 18–29% for the detection rate of VT (1 4 7) Generally with previous monitoring methods the detection rate of clinically significant episodes of VT was probably higher than that for all kinds of VT episodes However in the present study the conventional HR dependent alarm system which worked in parallel with the automated system was only activated in two out of 44 cases of VT as compared to 17 out of 24 instances of asystole Thus it appears that the introduction of automated monitoring in the CCU has specifically improved the detection rate of VT Julian and Vetter (7) using a special arrhythmia detector for ECG monitoring from one patient at a time reported a very high performance in VT Their detector however could distinguish only roughly between different arrhythmias and was also used to establish the true VT frequency Such a method may result in overestimation of the performance of the detector

When the number of patients with asystole VF and VT was estimated during the 4 week period by independent analysis of the continuous ECG and the computer generated write-outs the numbers were the same for asystole and VF whereas VT was seen in 19 patients in the continuous ECG records compared with 18 in the write-outs With the combination of both methods however VT was found in 21 out of 65 patients since one VT was overlooked in the manual interpretation and a few others occurred when the ECG recordings were interrupted At present most Swedish cardiologists define VT as three or more VBs in succession with a frequency above 100 (1 4) It was felt however that such limits would result in too high a number of VT messages reducing the respect of the nursing staff for the highest priority alarms Preferably the tachycardia limit should be the same in ordinary rhythm as in VT and SVT We also aimed to reduce the number of lowest priority tachycardia alarms For these reasons a tachycardia limit of 120 was chosen At what point antiarrhythmic treatment should be instituted when runs of VBs occur in AMI has not yet been determined Such treatment is given in order to improve haemodynamics and prevent the occurrence of VF From a practical point of view however a therapy-directed definition of ventricular tachycardia would be advantageous

In the computer generated write-outs the ECG and the diagnostic message were presented in parallel This might have introduced some bias into the results from the 6-month period During the 4 week period however ECG interpretation was performed without knowledge of the computer diagnosis In the latter study the write-outs were not analysed until the whole material had been collected and the interpretation of the two materials by the physician differed in only one case of true VF diagnosed as VT in the write-outs An exact manual interpretation of a single lead ECG is sometimes impossible and uncertainty regarding the interpretation was noted in 34 arrhythmia episodes from 13 patients during the 6-month period All but three of these episodes were reported as VT by the computer and were therefore included in the figures as false positive VT alarms Only a few questionable beats were noted in patients with atrial fibrillation and thus aberrant conduction in this arrhythmia did not usually cause any special diagnostic problems About 2/3 of all false positive VT alarms occurred in patients with atrial fibrillation or flutter This supraventricular arrhythmia was observed in 20% of the patients and about 80% of these were free from false positive VT alarms A prolonged QRS interval was seen in all cases of false positive VF alarms except those caused by artefacts or high P or T waves in the ECG However 85% of the patients with BBB had no false positive VF alarms Thus in routine monitoring the system worked satisfactorily in the majority of patients with atrial fibrillation (flutter) and/or BBB

In the inquiry all 13 nurses involved considered that working conditions in the unit improved after the introduction of the automated monitoring

system However one nurse also thought that the high number of arrhythmias detected had a negative effect by increasing demands on the staff Five out of 10 nurses thought that patient care had improved with the system and the rest considered it un changed Eight out of 11 considered the ratio of false to true alarms of all kinds to be acceptable or low whereas three thought this ratio too high Of course no definite conclusions as to the acceptabil ity of the system or the opinion of the nurses as to the highest priority alarms can be drawn from the results of the inquiry It was found however that the VB detecting function had been turned off in a small number of cases Reasons for such an action have been a high number of true or false highest priority alarms or false alarms due to intermittent external pacemaker activity Naturally the above procedure has somewhat decreased the number of false VT or VF alarms as well as the number of true VT alarms during the 6-month period Moderately *frequent false highest priority alarms (1–5 per hour* for a few hours) in the occasional patient (2–3 per month) have been accepted by the nurses

Automated ECG monitoring makes it possible to analyse arrhythmia frequency and incidence in large materials Probably this will improve knowledge of the significance of heart rhythm dis turbances in CCU patients

ACKNOWLEDGEMENTS

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REFERENCES

1 Holmberg S Ryden L & Waldenstrom A Effi ciency of arrhythmia detection by nurses in a coronary care unit using a decentralised monitoring system Br Heart J 39 1019 1977
2 Hulting J Blomqvist P & Nygårds M E Computer based ECG analysis in acute myocardial in farction A comparison between two computer pro- grams for the detection of ventricular arrhythmias Acta Med Scand 201 439 1977
3 Hulting J & Nygårds M E Accuracy of alarms from a computer-based arrhythmia monitoring system Acta Med Scand 203 153 1978
4 Mogensen L Ventricular tachyarrhythmias and lignocaine prophylaxis in acute myocardial infarction A clinical and therapeutic study Acta Med Scand (Suppl) 513 1970
5 Nygårds M E & Hulting J Recognition of ventricu lar fibrillation utilizing the power spectrum of the ECG Computers in Cardiology IEEE Computer Society 1977
6 — A system for automated ECG monitoring Comput Biomed Res Submitted for publication 1978
7 Vetter N J & Julian D G Comparison of arrhyth mia computer and conventional monitoring in coronary-care unit Lancet 1 1151 1975

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Evolution of ST Segment and Q and R Waves during Early Phase of Infenor Myocardial Infarction

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ABSTRACT Using the aVF lead the ST segment deviation and the amplitudes of Q and R waves were measured hourly for 48 hours after the onset of symptoms in 14 patients with inferior AMI The average ST deviation reaches a maximum elevation at about 3 hours and then falls to a uniform level within 12 hours The average depth of the Q waves began to increase within the first 2 hours, and the increase was linear until around the 12th hour, whereafter the depth was somewhat uneven The average height of the R waves declined almost linearly from the 3rd to 15th hour after which the height became fairly uniform Within a 48-hour period after the onset of symptoms, there was a significant positive correlation not only between the average amplitudes of the Q and R waves ($r=0\ 951$, $p<0\ 001$), but also between the courses of the individual Q and R waves in 13 of the 14 patients A significant positive correlation was found between ST elevation at 3 hours and the reduction in the R wave within 24 hours ($r-0\ 634$, $p<0\ 05$) but no correlation existed between ST elevation and the Q wave at these times It is concluded that within 48 hours of the onset of symptoms the magnitude of electrically inert myocardial tissue can be determined by the course of the Q or R waves but that during the first 24 hours the R wave is superior to the Q wave for evaluating the evolution of the infarction process

Key words ST segment amplitude of Q and R waves inferior AMI

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Characteristic changes in the QRS complex and the ST segment occur during the progression of myocardial infarction (2) The appearance of a Q wave and reduction in height of the R wave are taken as an expression of a loss of electrically active myocardium (5 7 15 18) ST elevation is presumed to reflect severe myocardial ischaemia with injury to the cell and its membrane (9 10) but the electrophysiological basis of changes in the ST segment is still not completely clarified (12)

Recent investigations suggest that the most pronounced development in the infarction process in man takes place within the first 24 hours after the onset of symptoms (1 13 14) This makes it essential to know the exact time sequence of the electrocardiographic (ECG) changes in the early phase of acute myocardial infarction (AMI) With this in mind we have recorded the ECG every hour for 48 hours in a selected group of patients in whom the first ECG was obtained within 4 hours of the onset of symptoms Thereafter the spontaneous course of the Q and R waves as well as of the ST segment were plotted and an attempt was made to relate the early ST elevation to changes in both the Q and R waves

PATIENTS AND METHODS

The investigation comprises 14 patients with inferior (diaphragmatic) AMI whose first ECG recording taken ≤240 min after the onset of symptoms showed an ST elevation of ≥1 mm in leads III and /or aVF The patients were admitted to the Coronary Care Unit (CCU) of the Odense University Hospital on average 96 min (range 25–210) after the onset of symptoms (i e retrosternal pain of more than 20 min duration) Their average age was 63 years (range 44–75) 10 were men and 4 women Thirteen patients had their first AMI one patient had suffered from anterior infarction 10 years earlier Patients with stethoscopic signs of pericarditis disturbances of electrolyte balance a QRS of≥0 12 sec or patients on drug therapy possibly affecting the ST segment (digitalis quinidine isoproterenol verapamil β adrenergic blocking drugs) were not admitted to the study

The patients were treated in accordance with the usual procedure of the CCU i e rest in bed morphine antiarrhythmic drugs and diuretics as required and oxy

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Abbreviations ECG=electrocardiogram AMI=acute myocardial infarction CCU=coronary care unit

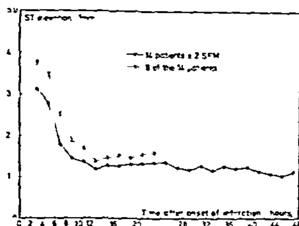


Fig. 1. Mean ST elevation at intervals of 2 hours from the onset of symptoms of AMI (aVF lead).

gen via a nasal catheter when necessary. Stethoscopy of the lungs and heart was carried out frequently. A chest X-ray was taken if stethoscopy suggested congestive heart failure. Based on *the clinical classification of congestive* heart failure in patients with AMI (19) one patient had no signs of cardiac failure, 12 patients had mild to moderate heart failure with rales of both lung fields of 50% or less and one patient pulmonary oedema with rales over more than 50% of both lung fields. Based on daily routine ECG recordings all 14 patients had according to WHO criteria (20) *unequivocal ECG changes expressed as development* of pathological Q waves in leads III and/or aVF.

ECG recording and measurement

ECG was recorded using the aVF lead immediately after admission to hospital and thereafter on the hour for 48 hours after the onset of symptoms. The recordings were performed with the patient in the supine position but with the upper part of the body at an angle of 30°, with Siemens-Elema Mingograph 34, paper speed 50 mm/sec, calibrated to 1 mV = 10 mm. The ECG complex was analysed by means of a Hewlett Packard 9864 A digitizer/9810 A calculator. The digitizer consists of three elements: a platen with a digitizing surface, a free moving cursor and a main frame; it enters co-ordinate data from graphic records into the calculator. The duration of Q and QRS complex, the amplitudes of Q, R and any QS waves, and the height of the ST segment 0.06 sec after the nadir of the last wave (usually the S wave in the QRS complex) were calculated using the mean of 3 consecutive ECG complexes. The PQ level was employed as the base line. The Q, R and QS waves were identified using Minnesota code criteria (11). The measurement error of a deviation from base line (interobservation variation) showed a standard deviation of less than 0.2 mm (12).

In the final calculations, measured values of the amplitudes of Q, R and QS waves, as well as of the ST segment deviations, were included only if the duration of the QRS complex was <0.12 sec. Digoxin treatment during the period under study was commenced in 2 patients; *the values of the ST segment deviations were thereafter* excluded. As a few ECGs were for practical reasons recorded either a few minutes prior to or after the hour, it became necessary to use intervals of 120 min. An average value was therefore calculated for the measured amplitudes of Q and R waves, as well as of the ST segment deviations for each 120-minute interval from 1 to 2880 min after the onset of infarction.

A significance level of 0.05 was chosen in the statistical analyses.

RESULTS

Fig. 1 shows the average ST segment elevation of the 14 patients during the 48 hours after the onset of infarction, depicted at two-hour intervals and as recorded by the aVF lead. The highest ST elevation was measured in the 2–4 hour interval. In the following 4 hours the ST elevation decreased rapidly and thereafter there was a slower decrease up to the 12–14-hour interval when it became fairly stable. There is considerable scatter in the descending part of the curve; this corresponds to a relatively marked individual variation with regard to the time at which the ST elevation began to decrease. In 8 of the 14 patients the first ECG recording was carried out within 2 hours of the onset of symptoms. In these patients the ST segment increased from the time of recording to a maximum in the 2–4 hour interval. It would therefore appear that the previously mentioned high average ST elevation in the 14 patients in the 2–4-hour interval represents the maximum ST elevation of our sample.

In Fig. 2 the average amplitudes of the Q waves are depicted at the same intervals as in Fig. 1. In 14 patients the Q wave increased in depth almost

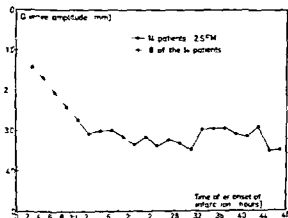


Fig. 2. Mean amplitude of Q wave at intervals of 2 hours from the onset of symptoms of AMI (aVF lead).

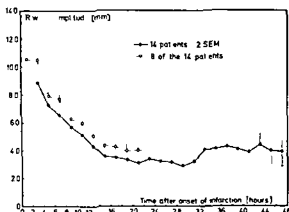


Fig 3 Mean amplitude of R wave at intervals of 2 hours from the onset of symptoms of AMI (aVF lead)

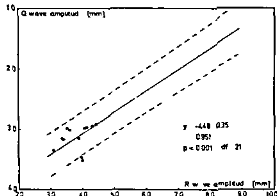


Fig 4 Correlation between the mean amplitudes of R and Q waves at intervals of 2 hours (95% confidence limits)

linearly from the 2–4-hour to the 12–14 hour interval In the following period the amplitude became somewhat erratic with a slight reduction of the depth from the 32 to the 44 hour interval The scatter on the descending linear part of the curve was rather small corresponding to a uniform initial change in the Q wave in all 14 patients but after 12 hours there was considerable scatter indicating a marked individual variation in the depth of the Q wave In the ECGs of 8 patients which were recorded early a rise in the amplitude of the Q wave was observed already during the 0–2 hour interval indicating that the development of the Q wave begins within 2 hours of the onset of infarction The duration of the Q wave of all 14 patients became ≥0 03 sec during the 48-hour period QS complexes occurred in 3 patients after 21 18 and 11½ hours respectively

Fig 3 shows the average amplitude of the R waves during a period of 48 hours after the onset of infarction in the 14 patients depicted at intervals of 2 hours and recorded by the aVF lead The amplitude of the R wave decreased almost linearly from the 2–4-hour to the 14–16-hour interval In the following period the amplitude was at a fairly uniform level with a slight rise after 32 hours The scatter was rather constant throughout the 48 hours corresponding to an almost uniform course of the height of the R wave for all 14 patients In the ECGs of 8 patients recorded early it was observed that the height of the R wave was constant during the first 2 intervals suggesting that the R wave begins to develop in the 2–4-hour interval

Fig 4 shows the relationship between the amplitudes of the Q and R waves in the 14 patients Comparison of the average amplitudes of both the Q and R waves in comparable 2 hour intervals within the 48 hours after the onset of infarction demonstrated a highly significant linear correlation (r=0 951 p<0 001)

The correlation between the amplitudes of the Q and R waves of each patient between comparable 2 hour intervals within the 48 hours after the onset of infarction is shown in Table I A significant positive linear correlation could be observed in 13 of the 14 patients

ΔST was calculated for each patient as the difference between the ST elevation at 3 hours (average ST elevation between 121 and 240 min) and 24

Table I *Correlation between the amplitudes of R and Q waves at intervals of 2 hours*

Pat no	*r*	*n*	*p*
1	0 940	23	<0 001
2	0 657	19	<0 01
3	0 939	18	<0 001
4	0 823	23	<0 001
5	0 634	16	<0 01
6	0 774	22	<0 001
7	0 836	14	<0 001
8	0 528	19	<0 05
9	0 328	23	N S
10	0 488	23	<0 05
11	0 672	23	<0 001
12	0 699	23	<0 001
13	0 783	21	<0 001
14	0 593	23	<0 01

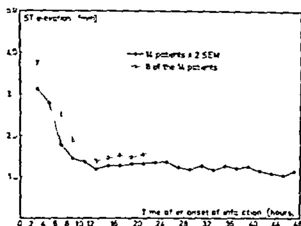


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In the final calculations measured values of the amplitudes of Q R and QS waves as well as of the ST segment deviations were included only if the duration of the QRS complex was ≤0 12 sec Digoxin treatment during the period under study was commenced in 2 patients the values of the ST segment deviations were thereafter excluded As a few ECGs were for practical reasons recorded either a few minutes prior to or after the hour it became necessary to use intervals of 120 min An average value was therefore calculated for the measured amplitudes of Q and R waves as well as of the ST segment deviations for each 120-minute interval from 1 to 2880 min after the onset of infarction

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In Fig 2 the average amplitudes of the Q waves are depicted at the same intervals as in Fig 1 In 14 patients the Q wave increased in depth almost

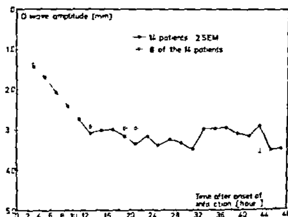


Fig 2 Mean amplitude of Q wave at intervals of 2 hours from the onset of symptoms of AMI (aVF lead)

the myocardial tissue becomes electrically inert and that the extent of this can be determined either by measuring the increasing depth of the Q wave or the reduction in height of the R wave

It has been demonstrated experimentally that the elevation of the ST segment 15 min after occlusion predicts the changes in the Q and R waves 24 hours later (4) The present investigation could confirm this with regard to the R wave inasmuch as a significant positive correlation was found between the elevation of the ST segment at 3 hours and the reduction in the R wave within 24 hours after the appearance of infarction whereas no corresponding correlation could be demonstrated between ST elevation and development of the Q wave The lack of correlation is possibly due to the fact that the Q wave does not develop to its maximal extent within the given period Therefore it would appear that the R wave is more reliable during the first 24 hours for *predicting the course of the infarction* This assumption is supported by a study of Heng et al (3) who were unable to demonstrate any reliable relationship between the early ST elevation and the development of the Q wave within the first 24 hours after experimental coronary artery occlusion

CONCLUSION

Within 48 hours of the onset of symptoms the magnitude of electrically inert myocardial tissue can be determined from the course of the Q and R waves but during the first 24 hours the R wave is superior to the Q wave for evaluating the evolution of the infarction process

ACKNOWLEDGEMENT

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REFERENCES

1 Askenazi J Maroko P R Lesch M & Braunwald E Usefulness of ST segment elevation as predictors of electrocardiographic signs of necrosis in patients with acute myocardial infarction Br Heart J 39 764 1977

2 Goldman M J Principles of clinical electrocardiography Lange Medical Publications Los Altos 1976

3 Heng M K Singh B N Norris R M John M B & Elliot R Relationship between epicardial ST segment elevation and myocardial ischemic damage after experimental coronary artery occlusion in dogs J Clin Invest 58 1317 1976

4 Hillis L D Askenazi J Braunwald E Radvany P Muller J E Fishbein M C & Maroko P R Use of changes in the epicardial QRS complex to assess interventions which modify the extent of myocardial necrosis following coronary artery occlusion Circulation 54 591 1976

5 Johnston F D Hill I G W & Wilson F N The form of the electrocardiogram in experimental myocardial infarction II The early effects produced by ligation of the anterior descending branch of the left coronary artery Am Heart J 10 889 1935

6 Maroko P R Kjekshus J K Sobel B E Watanabe T Covell J W Ross J Jr & Braunwald E Factors influencing infarct size following experimental coronary artery occlusion Circulation 43 67 1971

7 Myers G B Klein H A & Hiratzka T V Correlation of electrocardiographic and pathologic findings in posterior infarction Am Heart J 38 547 1949

8 Poliwoda H The thrombolytic therapy of acute myocardial infarction Angiology 17 528 1966

9 Prinzmetal M Toyoshima H Ekmekci A Mizuno Y & Nagaya T Myocardial ischemia Nature of ischemic electrocardiographic patterns in the mammalian ventricles as determined by intracellular electrographic and metabolic changes Am J Cardiol 8 493 1961

10 Rakita L Borduas J L Rothman S & Prinzmetal M Studies on the mechanism of ventricular activity XII Early changes in the RS T segment and QRS complex following acute coronary artery occlusion Experimental study and clinical applications Am Heart J 48 351 1954

11 Rose G A & Blackburn H Cardiovascular survey methods WHO Monogr Ser 56 1968

12 Ross J Jr Electrocardiographic ST segment analysis in the characterization of myocardial ischemia and infarction Circulation (Suppl) I 73 1976

13 Selwyn A P Ogunro E A & Shillingford J P Natural history and evaluation of ST segment changes and MB CK release in acute myocardial infarction Br Heart J 39 988 1977

14 — Loss of electrically active myocardium during anterior infarction in man Br Heart J 39 1186 1977

15 Shaw C McK Jr Goldman A Kennamer R Kimura N Lindgren I Maxwell M H & Prinzmetal M Studies on the mechanism of ventricular activity VII The origin of the coronary QR wave Am J Med 16 490 1954

16 Thayssen P Nielsen J S & Nielsen B L Magnitude and duration of ST segment elevation in patients surviving their first acute anterior or inferior myocardial infarction Dan Med Bull 22 120 1975

17 Thygesen K Hørder M Nielsen B L & Petersen P H The variability of ST segment in the early phase of acute myocardial infarction In New aids in diagnosing acute myocardial infarction (ed B W Johansson) pp 61-70 Acta Med Scand (Suppl) 623 1979

18 Wilson F N Hill I G W & Johnston F D The form of the electrocardiogram in experimental myocardial infarction III The later effects produced by ligation of the anterior descending branch of the left coronary artery Am Heart J 10 903 1935
19 Wolk M J Scheidt S & Killip T Heart failure complicating acute myocardial infarction Circulation 45 1125 1972
20 Working group on the establishment of ischaemic heart disease registers Report of the fifth working group WHO Euro 8201 (5) Copenhagen 1971

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Effect of Amiodarone in the Wolff-Parkinson-White Syndrome

A Clinical and Electrophysiological Study

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ABSTRACT Six patients with Wolff Parkinson White (WPW) syndrome were given long term treatment with amiodarone Symptomatic relief was obtained in all Tolerance to the drug was good Reversible corneal changes appeared after some weeks treatment in five patients No thyroid side effects were noticed Prior to treatment dual atrioventricular (AV) conduction was demonstrated on His bundle electrograms in all six patients Recordings were made at varied heart rates using atrial and ventricular pacing Reciprocating tachycardia was readily provoked by properly timed extra stimuli in all patients When amiodarone treatment had become clinically effective, a second comparative study was made in four patients after 26–85 days treatment Amiodarone reduced heart rate and second degree AV block appeared at a lower atrial pacing rate It increased the refractory periods of right atrium AV node and the accessory pathway in proportion to the duration of treatment Induction of tachycardia was effectively prevented by the drug It appears that amiodarone in chronic treatment has a *predictable* and unique depressant action on cardiac conduction, supporting the opinion that this compound despite side-effects has an *important role to* play in the treatment of refractory arrhythmias in patients with the WPW syndrome

Key words Amiodarone WPW syndrome His bundle study reciprocating tachycardia

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The arrhythmias associated with the Wolff Parkinson White (WPW) syndrome appear to be more serious than originally believed (16) Many reports on total disablement due to tachycardia have been published and sudden unexpected death ›as convincingly correlated to the syndrome in a umber of cases (10 11 14 17 18 20) Drugs lable up to this time have proved ineffective in rolling tachyarrhythmias of many patients Furthermore undesired and even potentially hazardous effects on cardiac conduction were revealed in some of these patients (12 39)

In recent years development of a safe technique for electrophysiological studies in man has made possible a more aggressive approach to therapy (11 33 37) As the anatomic site and the functional role of accessory pathways (AP) can now be demonstrated with reasonable certainty (27 30 34) surgical interruption of the structures constituting the re-entry circuit seems to be a rational therapeutical procedure which was successfully carried out in a number of cases (3 5 7 29 33 37) However reports do not reflect a unanimous optimism as results are difficult to predict in many cases and as the technical procedures are long lasting and complex (17 37)

Recently a promising alternative treatment of the WPW syndrome has become available with the introduction of amiodarone Thus Rosenbaum et al (23 24) found a convincing clinical effect during chronic treatment Earlier electrophysiological studies however do not unequivocally explain this action probably because the effect of amiodarone was tested in acute experiments using i v administration (4) and after peroral treatment for only two weeks (38)

The purpose of this study is to provide further documentation of the unique effect of amiodarone in WPW syndrome with special emphasis on the

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Abbreviations A=atriogram D=delta wave of QRS complex H=His potential V=ventriculogram AH=interval between A and H AP=accessory pathway AV atrioventricular HV=interval between H and V RA=right atrium SA=sinoatrial AVN AV node ERP=effective refractory period FRP=functional refractory period HBE=His bundle electrogram SAN=SA node WPW=Wolff Parkinson White

Table I *Clinical and ECG features of the patients*

Pat no	Age (y)	Sex	Symptoms	ECG: Type of WPW syn drome	ECG: Ventricular rate during spontaneous tachycardia (beats/min)	ECG: Delta wave present during tachy cardia
1	24	♂	Frequent enduring palpitations for several years Periodically invalided by rapid arrhythmias refractory to digoxin prac tolol verapamil in varying doses and combinations Frequent DC conversions necessary several times complicated by ventricular fibrillation Syncope once and minor cerebral symptoms several times Fatigue constantly present during arrhythmias	A	135 200	+ ++
2	66	♂	Frequent episodes of lightheadedness and nearsyncope over a couple of years Extreme fatigue and anxiety during tachy cardia	B	165	–
3	40	♂	Frequent palpitations lightheadedness and nearsyncope over a few years Extreme anxiety and fatigue during tachycardias	B	Not verified electrocardio graphically	
4	29	♂	Frequent palpitations since early adoles cence	A	200	–
5	19	♀	Attacks of palpitations with increasing frequency over two years Several times minor cerebral symptoms Extreme fatigue during tachycardia	B	200 150 (atrial flutter with 2 1 block)	– +
6	39	♂	Frequent palpitations since childhood	B	210	

electrophysiological changes induced by the drug after several weeks treatment Clinical and electrophysiological data are presented on six pa tients in whom a control His bundle study was made prior to long term treatment with amiodarone In four cases a second comparative study was performed when treatment had been clin ically effective for at least two weeks

PHARMACOLOGY OF THE DRUG

Amiodarone hydrochloride (2-n butyl 3-(4 beta-diethyl aminoethoxy 3 5 diiodobenzoyl) benzofuran hydrochlo ride) is a benzofuran derivative structurally resembling thyroxine Originally introduced as an antianginal drug it was later shown to possess unique antiarrhythmic prop erties Briefly the drug produces an atropine resistant bradycardia dilates coronary vessels and depresses myocardial oxygen consumption without having a significant effect on cardiac output The drug belongs to class III of the classification of antiarrhythmic drugs by Vaughan Williams (32)

The ECG shows a prolonged QT_c (2 8) Olsson et al recording monophasic right atrial potentials in man showed that amiodarone prolongs the repolarization phase (19) The drug antagonizes several effects of sympathetic stimulation

A hypothyroid state is produced in rabbit heart muscle without adverse extracardiac hypothyroid reactions i e a significant increase in the duration of the action potential of atrial and ventricular muscle is observed without an effect on the resting membrane potential or the amplitude and the maximal rate of depolarization of the action poten tial (21 26)

SUBJECTS AND METHODS

The study group consisted of six patients with WPW syndrome Their clinical and ECG features are sum marized in Table I All patients were treated with amiodarone in doses of 200–600 mg daily Initially 600 mg/day were given for 10–14 days followed by 400 mg/day until clinical effect was obtained when the dose was reduced to the minimum sufficient to suppress attacks of tachycardia 250–400 mg a day

The patients were followed for periods of 8–28 months Clinical examination was made and standard ECG was recorded at short intervals during the initial two months of

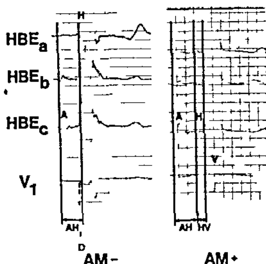


Fig 1 His bundle electrograms (*HBE*) and simultaneous external lead from patient 3 before (*AM–*) and during (*AM+*) amiodarone treatment V =precordial lead

treatment Later the patients were seen in the Cardiological Department every three months or earlier if tachycardia reappeared Ophthalmological inspection was made at intervals of 3–4 months Plasma thyroxine was measured prior to treatment and thyroid function was evaluated at check ups

After informed consent had been obtained a His bundle study was performed in all six patients prior to amiodarone treatment at a time when no medical therapy was given In four cases a second comparative study was made when clinical effect had been obtained 76–85 days after prescription of the drug

The His bundle studies were performed as originally described by Scherlag et al (25) by introduction of a multipolar electrode catheter via the right femoral vein to the right side of the heart The catheter tip was positioned across the tricuspid valve in close proximity to the atrial septum Further catheters were introduced for right atrial and ventricular pacing which was performed at increasing rates and using the extra stimulus technique (6 31) Refractory periods were determined using the lowest possible overdrive pacing rate sufficient to cause a constant suppression of the sinoatrial node (SAN) Tachycardia was provoked and terminated by right atrial or right ventricular extra stimuli at varying coupling intervals The following intervals were measured SAN recovery time longest interval between two succeeding atriograms (AA) in the first three cycles following cessation of rapid atrial pacing at varying rates AH interval interval from the first rapid deflection of the atriogram (A) to the first rapid deflection of the His potential (H) HV interval interval from the first rapid deflection of H to the QRS onset on the ECG (Figs 1 and 2) The effective refractory period of right atrium (ERP_{RA}) the longest coupling interval of paired pace impulses at which the extra stimulus did not provoke atrial depolarization The effective refractory period of the atrioventricular node (ERP_{AVN}) the longest coupling interval of paired pace impulses at which the extra stimulus failed to conduct to the bundle of His The functional refractory period of the AVN (FRP_{AVN})=the shortest interval between two consecutive His potentials both of which were propagated from right atrium during paired pacing The effective refractory period of AP (ERP_{AP}) the longest coupling interval of paired pace impulses at which no delta wave was detectable in the QRS complex of the premature beat (Fig 3)

All recordings were made on a six-channel ECG recorder (Mingograph 81 Siemens Elema) The sensitivity was 200 µV/cm and the frequency band used was 50–700 Hz The equipment was carefully earthed at one point only so as to avoid ground loops A switch box connecting the intracavitary electrodes and the recording equipment had a built in current limiter (20 µA in case the patient was touching 220 V a c) The recordings were made at a paper speed of 100 mm/sec

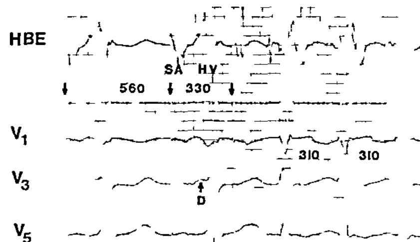


Fig 2 Tracings from patient 5 during extra stimulus pacing Arrows indicate pace stimuli A tachycardial episode is provoked by the third stimulus V1 V3 V5 precordial leads

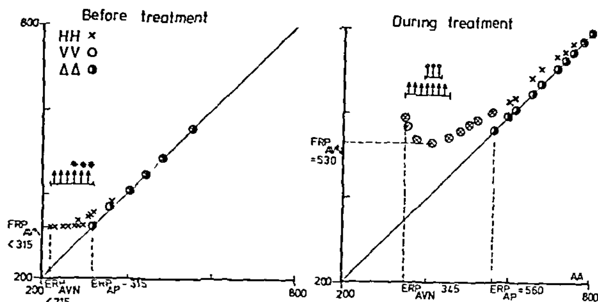


Fig 3 Response of AV conduction to atrial extrasystoles of increasing prematurity introduced during continuous right atrial pacing in patient 5 before and during chronic treatment with amiodarone All values are given in ms *AA* = coupling interval of right atrial extrasystole *HH* = interval between corresponding His potentials VV = interval between corresponding ventriculograms ΔΔ = intervals between corresponding delta waves ↑↑↑ = trigger zone ♀ = atrial echo * = tachycardial episode

Statistical analyses were performed using Student s *t* test of paired data comparing electrophysiological parameters before and during amiodarone treatment

RESULTS

5 patients obtained complete and one patient partial symptomatic relief after total doses of 14–20 g amiodarone over 3–4 weeks Maintenance dose was 250–400 mg/day The patient with only partial relief had hypertensive heart disease and complained of vague discomfort probably not related to amiodarone treatment No intolerance was reported by the other five patients Corneal deposits were discovered in five patients after 17–34 weeks of

Table II *Results of His bundle studies in four patients with WPW syndrome before (AM−) and during long term treatment (AM+) with amiodarone*

Measures are given in ms Negative HV intervals indicate presence of delta wave

Case no	Days of treatment prior to 2nd study	SAN rate (beats/min)		SAN recovery time (<128 % of SAN rate)		AH interval		HV interval		Lowest pacing rate causing 2nd degree AV block (beats/min)		ERP_{RA}	
		AM−	AM+	AM−	AM+	AM−	AM+	AM−	AM+	AM−	AM+	AM−	AM+
1	85	65	60	–	1 160	60	100	55	55	200	135	–	255
3	26	70	65	1 200	1 210	130	130	−20	45	155	130	295	290
5	78	80	60	1 200	1 520	105	120	−10	−10	210	125	215	300
6	34	80	65	1 330	1 470	85	85	−10	−10	–*	170	240	250
Mean ± S E M													
AM−		74+3		1 243±13		95±15				188±15		250±24	
AM+		63±2		1 400±96[b]		109±10				133±3[b]		290±6[b]	
P value		0 01		n s		n s				0 05		0 05	
Normal values						85–140		35–55					

* Determination impaired by tendency to tachycardia
[b] Mean of 3 patients only

Table III *Results of His bundle studies in two patients with WPW syndrome before treatment with amiodarone*

Case no	SAN rate (beats/min)	SAN recovery time (128% of SAN rate)	AH interval	HV interval	Lowest pacing rate causing 2nd degree AV block (beats/min)	ERP_{RA}	ERP_{AP}	ERP_{AVN}	FRP_{AVN}
2	80	1 300	105	50	–	190	450	290	355
4	85	1 085	120	–15	Appr 160	190	440	265	290

Normal values given in Table II

treatment No case of photosensitivity was seen Thyroid function remained normal during treatment in all patients

The results of the electrophysiological studies are presented in Tables II and III Prior to treatment existence of dual AV conduction was demonstrated in all patients using incremental atrial pacing and episodes of reciprocating tachycardia were readily provoked by properly timed atrial or ventricular extra stimuli In the second comparative study which was performed in four of the patients tachycardia could no longer be induced

Sinoatrial node and RA

Sinus rate was reduced in all four patients examined twice Heart rate (mean ± S E M) was 74±3 beats/min before and 63±2 during treatment ($p<0\ 01$) SAN recovery time (mean ± S E M) compared in

RP$_{AP}$		ERP_{AVN}		FRP_{AVN}	
M–	AM+	AM–	AM+	AM–	AM+
95	∞	≤250	360	335	520
0	∞	310	370	425	480
15	560	≤215	345	315	530
	310	260	310	360	405
		259±19		359±23	
		346±13		484±28	
		0 01		0 01	
		230–390		330–550	

three patients was 1243±13 ms before and 1400±96 during treatment (not significant) ERP_{RA} (mean ± S E M) was 250±24 ms before and 290±6 during treatment ($p<0\ 05$)

AVN conduction

AH interval (mean ± S E M) in all six patients before treatment was 101±10 ms In the four patients examined twice no significant change in AH interval was induced by amiodarone However in two patients (nos 1 and 5) who had been treated for nearly three months at the time of the second study a marked prolongation of the AH interval was found which was absent in the other two patients who had received only four weeks treatment prior to the second study

Lowest pacing rate (mean ± S E M) causing second degree AV block was 188±15 beats/min before and 133±3 during treatment ($p<0\ 05$) ERP_{AVN} (mean ± S E M) was 275±14 ms in all six patients prior to treatment The effect of amiodarone was estimated in four ERP_{AVN} (mean ± S E M) was 259±19 ms before and 346±13 during treatment ($p<0\ 01$) Respective values of FRP_{AVN} were 347±18 ms in the whole group prior to treatment 359±23 ms before and 484±28 ms during treatment in the four patients studied twice ($p<0\ 01$)

Intraventricular conduction

HV intervals could be measured with reasonable certainty in only two patients as prominent delta waves slurred the ventricular complexes All values obtained were within normal limits

Paranodal conduction via AP

ERP_{AP} (mean ± S E M) of antegrade conduction was 368±36 ms in five patients prior to treatment In two cases the AP was fully suppressed at the

Table IV *Effect of drugs on dual AV pathways in the WPW syndrome*

	ERP_{AVN}	ERP_{AP}	HV	
Quinidine and compounds with a quinidine like action	0	↗	↗	*
Beta blocking agents	↗	0	0	*
Digoxin and other digitalis derivatives	↗	↙	0	*
Verapamil	↗	↙ (0)	0	*
Amiodarone	↗	↗	↗	

* Based on data from Wellens in Cardiac arrhythmias (1975)

time of the second study In one patient ERP_{AP} was prolonged by 78% In one case determination was impossible as tachycardia constantly arose during extra stimulus pacing

DISCUSSION

The present study revealed that amiodarone in long term treatment i e four weeks or more markedly reduced sinus rate and prolonged refractory periods of RA AVN and AP in patients with dual AV conduction These observations are in keeping with a recent report by Wellens et al (38) and *Coumel and Bouvrain (4) but unlike Wellens et al* [illegible] unable to provoke tachycardial episodes in [illegible]arative studies during treatment This dif[illegible]e might be related to the time at which the second study was carried out *In the series of* Wellens et al all patients were studied at intervals of 14 days between the first and second study whereas our patients were treated much longer (26–85 days) before the comparative study

In the present series all patients had to be treated for 3–4 weeks before full clinical effect was obtained and the electrophysiological studies likewise suggest that tissue saturation of amiodarone does not reach its maximum until several weeks have passed

The uniform depressant action on both specialized and ordinary myocardial cells appears to be a property unique to amiodarone Thus quinidine and other compounds with a quinidine like effect do not always depress AVN conduction (15 36) β blocking agents have no significant effect on conduction in *ordinary myocardial cells* (22 39) and digoxin and other derivatives of digitalis may increase conduction capacity of accessory pathways (35 39) a property shared by verapamil (12 28) (Table IV) These effects represent a potential danger as re entry facilitation and hence increased tendency to tachycardia may be the result or rapid tachyarrhythmias may be transmitted more easily to the ventricles

Corneal deposits almost invariably appear in adults during treatment with amiodarone but visual disturbances are rare and irreversible consequences of the corneal precipitates have not hitherto been reported *Also thyroid disturbances have* been reported in a few cases (9 13) At present these complications appear to be reversible (1 9)

The present study confirms the favourable clinical *response to amiodarone in the WPW syndrome* and demonstrates that the drug exerts a uniform depressant action on cardiac tissue which cannot be obtained with certainty using other presently available compounds singly or combined In our opinion the drug has a definite role to play in the treatment of patients with the WPW syndrome although its use at the present time should be restricted to cases resistant to other medical agents and rigorous control is necessary until more experience of thyroid and other side effects has been gathered during long term therapy

REFERENCES

1 Burger A Dinichert D Nicod P Jenny M Lemarchand Beraud T & Vallotton M B Effect of amiodarone on serum triiodothyronine reverse triiodothyronine thyroxin and thyrotropin J Clin Invest 58 255 1976
2 Charlier R Deltour G Baudine A & Chaillet F Pharmacology of amiodarone an antianginal drug with a new biological profile Arzneim Forsch 11 1408 1968
3 Cobb F R Blumenschein S D Sealy W C Boineau J P Wagner G S & Wallace A G Successful surgical interruption of the bundle of Kent in a patient with Wolff Parkinson White syndrome Circulation 38 1018 1968
4 Coumel P & Bouvrain Y Etude clinique des effets pharmacodynamiques de l amiodarone J Agreges 6 69 1973
5 Dreifus L S Nichols H Morse D Watnabe Y & Truex R Control of recurrent tachycardia of Wolff Parkinson White syndrome by surgical ligature of the a v bundle Circulation 38 1030 1968
6 Durrer D Schoo L Schuilenburg R M & Wellens H J J The role of premature beats in the initiation and the termination of supraventricular tachycardia in the Wolff Parkinson White syndrome Circulation 36 644 1967

7 Edmonds J H Ellison R G & Crews T L Surgically induced atrioventricular block as treatment for recurrent atrial tachycardia in Wolff Parkinson White syndrome Circulation (Suppl) I 39 1 1969
8 Facquet J Milet M Grosgogeat Y Alhomme P & Vachon L L influence de l amiodarone sur le rythme cardiaque d electrocardiogramme Therapie 25 335 1970
9 Fidelle J E Attuel P Toumieux M C & Coumel P L utilisation de l amiodarone dans les troubles rythmiques graves et rebelles de l enfant Ann Cardiol Angeiol 5 385 1976
10 Flensted Jensen E Wolff Parkinson White syndrome A long term follow up of 47 cases Acta Med Scand 186 65 1969
11 Gallagher J J Gilbert M Svenson R H Sealy W C Kasell J & Wallace A G Wolff Parkinson White syndrome The problem evaluation and surgical correction Circulation 51 767 1975
12 Heng M K Singh B N Roche A H G Norris R M & Mercer C J Effects of intravenous verapamil on cardiac arrhythmias and on the electrocardiogram Am Heart J 90 487 1975
13 Jonckheer M H Broeckaert I Blockx P & Bernard R Amiodarone et fonction thyroidienne Arch Mal Coeur 69 1315 1976
14 Kimball J L & Burch G The prognosis of the Wolff Parkinson White syndrome Ann Intern Med 27 239 1947
15 Mandel W J Laks M M Obayashi K Hayakawa H & Daley W The Wolff Parkinson White syndrome pharmacologic effects of procaine amide Am Heart J 90 744 1975
16 Mortensen V Nielsen A L & Eskildsen P Wolff Parkinson and White s syndrome Acta Med Scand 118 506 1944
17 Narula O A Wolff Parkinson White syndrome A review Circulation 47 872 1973
18 Ohnell R F Pre-excitation A cardiac abnormality Acta Med Scand (Suppl) 152 115 1944
19 Olsson S B Brorson L & Varnauskas E Class 3 antiarrhythmic action in man Br Heart J 35 1255 1973
20 Onnius E Pre-excitation Studies on criteria prognosis and heridity Acta Med Scand (Suppl) 465 1966
21 Pritchard D A Singh B N & Hurley P J Effects of amiodarone on thyroid function in patients with ischemic heart disease Br Heart J 37 856 1975
22 Rosen K M Barwolf C Ehsani A & Rahimtoola S H Effects of lidocaine and propranolol on the normal and anomalous pathways in patients with preexcitation Am J Cardiol 30 801 1972
23 Rosenbaum M B Chiale P A Halpern M S Nau G J Przybylski J Levi R J Lazzari J O & Elizari M V Clinical efficacy of amiodarone as an antiarrhythmic agent Am J Cardiol 38 934 1976
24 Rosenbaum M B Chiale P A Ryba D & Elizari M V Control of tachyarrhythmias associated with the Wolff Parkinson White syndrome by amiodarone hydrochloride Am J Cardiol 34 215 1974
25 Scherlag B J Lau S H Helfant R H Berkowitz W D Stein E & Damato A N Catheter technique for recording His bundle activity in man Circulation 39 13 1969
26 Singh B N & Vaughan Williams E M The effect of amiodarone (a new antianginal drug) on cardiac muscle Br J Parmacol 39 657 1970
27 Spurrell R A J Problems concerning assessment of anatomical site of accessory pathway in the Wolff Parkinson White syndrome Br Heart J 37 127 1975
28 Spurrell R A J Krikler D M & Sowton E Effects of verapamil on electrophysiological properties of anomalous atrioventricular connexions in Wolff Parkinson White syndrome Br Heart J 36 256 1974
29 Svenson R H Gallagher J J Sealy W C & Wallace A G An electrophysiologic approach to the surgical treatment of the Wolff Parkinson White syndrome Circulation 49 799 1974
30 Svenson R H Miller H C Gallagher J J & Wallace A G Electrophysiological evaluation of the Wolff Parkinson White syndrome Problems in assessing antegrade and retrograde conduction over the accessory pathway Circulation 52 552 1975
31 Thomsen P E B Stendorff B & Gøtzsche H His bundt elektrokardiografi Ugeskr Laeger 137 1995 1975
32 Vaughan Williams E M The development of new antidysrhythmic drugs Schweiz Med Wochenschr 103 262 1973
33 Wallace A G Sealy W C Gallagher J J Svenson R H Strauss H C & Kasell J Surgical correction of anomalous left ventricular pre excitation Wolff Parkinson White syndrome (type A) Circulation 49 206 1974
34 Wellens H J J Contribution of cardiac pacing to our understanding of the Wolff Parkinson White syndrome Br Heart J 37 231 1975
35 Wellens H J J & Durrer D Effect of digitalis on atrioventricular conduction and circus movement tachycardias in patients with Wolff Parkinson White syndrome Circulation 47 1229 1973
36 Effect of procainamide quinidine and ajmaline in the Wolff Parkinson White syndrome Circulation 50 114 1974
37 Wellens H J J Janse M J van Dam R T van Capelle F J L Meijne N G Mellink H M & Durrer D Epicardial mapping and surgical treatment in Wolff Parkinson White syndrome type A Am Heart J 88 69 1974
38 Wellens H J J Lie K I Bar F W Wesdorp J C Dohmen H J Duren D R & Durrer D Effect of amiodarone in the Wolff Parkinson-White syndrome Am J Cardiol 38 189 1976
39 Wit A L Hoffmann B F & Rosen M R Electrophysiology and pharmacology of cardiac arrhythmias IX Cardiac electrophysiologic effects of beta adrenergic receptor stimulation and blockade Part C Am Heart J 90 795 1975

Effect of Oral Verapamil on Ventricular Irregularity in Long-Standing Atrial Fibrillation

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ABSTRACT In patients with chronic atrial fibrillation (AF) symptoms and cardiac function may be improved by regularizing the ventricular rhythm even though the AF persists This study concerned effects of i v and oral verapamil (V) on ventricular regularity A regularizing effect was observed in 5 out of 10 patients after 0 15 mg of V/kg b wt i v but in only one patient after 80 mg of V by mouth V in a dose of 240 mg by mouth resulted in ventricular regularity in 6 out of 10 other patients 320 mg in a further 2 and 400 mg in the remaining 2 patients Six patients were given chronic oral therapy in progressively increasing doses Although ventricular regularity and symptom relief were obtained, intolerable side-effects precluded the evaluation of subjective long term effects of this therapy in all but one patient Further investigations particularly concerning the pharmacokinetic mechanisms of V are needed before the treatment can be recommended for patients with chronic AF

Key words Atrial fibrillation verapamil ventricular rhythm

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Atrial fibrillation (AF) as such causes cardiac disability and haemodynamic disturbances (9) as well as an increased risk of systemic embolism For these reasons it is customary to endeavour to convert AF to sinus rhythm (SR) The accepted method of achieving SR in patients with AF is by means of direct current (DC) countershock However in most patients AF recurs within a few months and in some it is impossible to convert the patient to SR at all (8)

The impairment of left ventricular performance in patients with AF has been believed to be due not only to loss of atrial systole but also to irregularity of the RR interval (3) Ventricular function can thus be improved by regularizing the ventricular response in these patients Schamroth (11) demonstrated that i v injection of verapamil may result in a more regular ventricular rhythm even though the AF persists To our knowledge however the value of oral verapamil for ventricular regularization has not been well documented

The purpose of the present study was to assess the effect of oral verapamil on ventricular rhythm in patients with long standing AF

PATIENTS AND METHODS

To evaluate the effects of oral verapamil 20 patients (11 men and 9 women) with long standing AF who either relapsed to AF or could not be converted to SR with DC countershock were selected for this study Table I gives their pertinent clinical data and previous medication

The patients ages ranged between 44 and 79 years (mean 58) The duration of AF ranged between one month and 14 years Ten of the patients had valvular heart disease 1 thyrotoxic heart disease 4 hypertensive or arteriosclerotic heart disease and 5 lone AF All the patients had either grade II or grade III functional cardiac disability according to the New York Heart Association criteria None had clinical signs of left and/or right heart decompensation at the time of the test The main medication at the time of the trial was digitalis and dicoumarol in all patients β-adrenergic blocking drugs in 4 and verapamil 40–80 mg 3 times a day in 3

The patients were allowed to rest in the supine position for at least 20 min before electrocardiogram (ECG) recording A Mingograph ECG recorder was used for 2 min in patients 1–10 and 1 min in patients 11–20 at a paper speed of 100 mm/sec

For the study of the effect of verapamil the 20 patients were divided into two groups of 10 Patients 1–10 (group I) were given an i v injection of 0 15 mg/kg b wt of verapamil over a period of 2 min A 2 minute ECG was recorded before and immediately after the injection and at 10 20

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[1] Research fellowship from the Swedish National Association against Heart and Chest Diseases

Abbreviations AF=atrial fibrillation SR sinus rhythm DC=direct current ECG=electrocardiogram(s) BP blood pressure

Table I *Clinical details of the patients*

MS=mitral stenosis MIS=mitral insufficiency AS=aortic stenosis AI=aortic insufficiency MI=myocardial infarction MV=mitral valve

Case no	Age (y)	Sex	Diagnosis	Duration of AF (y)	Total heart vol (ml/m² BSA)[a]	Medication
1	69	♂	Lone AF	1	1200/620	Digoxin dicoumarol
2	62	♂	MS	2	1380/610	Digoxin furosemide+potassium chloride Dicoumarol
3	54	♀	MS	5	1000/560	Digoxin furosemide + potassium chloride Dicoumarol
4	44	♀	MS	4	1000/610	Digoxin furosemide + potassium chloride Dicoumarol
5	57	♀	MS MIS AS AI gout	11	850/560	Digoxin furosemide + potassium chloride Allopurinol dicoumarol
6	79	♀	*Hypertensive heart*	*14*	*1430/900*	Digoxin furosemide + potassium chloride
7	64	o	Lone AF grand mal epilepsy	6	930/550	Digoxin dicoumarol diphenyl hydantoin
8	57	♂	*AS AI MS MIS*	*11*	*910/500*	Digoxin furosemide + potassium chloride Dicoumarol
9	47	♂	MS	2	1100/520	Digoxin dicoumarol
10	58	♂	Old MI arteriosclerotic heart disease	10 mo	1510/760	Digoxin furosemide+potassium chloride Dicoumarol
11	60	♀	Floppy MV	5 mo	1070/690	Digoxin dicoumarol
12	63	♂	Lone AF	1	1140/600	Digitoxin
13[b]	55	♂	MS MIS	1	1030/590	Digoxin furosemide + potassium chloride Metoprolol dicoumarol
[illegible]	66	♂	Lone AF	Unknown	1100/620	Digoxin metoprolol dicoumarol
15[b]	50	♀	Hyperthyroidism (treated with radioactive iodine)	2	920/540	Digoxin dicoumarol
16	58	♂	MS MIS	1	1040/550	Digoxin verapamil dicoumarol
17[b]	56	♀	Hyperthyroidism (treated) MI 1974	2	620/390	Digoxin levothyroxin
18[b]	*50*	♀	*Operated MV disease*	*7*	*1370/770*	Digoxin *alprenol dicoumarol*
19	52	♀	Hypertension *Intermittent AF*	3	1060/550	Digoxin metoprolol verapamil
20	47	♂	Paroxysmal AF 1970	1 mo	1210/560	Digoxin dicoumarol verapamil

[a] Determined by the radiological method of Jonsell (4)
[b] Exercised

and 30 min after its completion Blood pressures (BP) were measured with a sphygmomanometer at all the above mentioned times and the patients were questioned about any subjective side-effects On the following day an oral test with 80 mg of verapamil was performed The patients were in the predigestive phase and the drug was given with a glass of water The ECG and BP were recorded before administration of the drug and at hourly intervals up to 3 hours after intake of the drug

Patients 11–20 (group II) were tested with a higher single oral dose of verapamil 240 mg The resting ECG and BP were recorded before the drug and at hourly intervals up to 6 hours after drug intake The patients who did not exhibit regular ventricular activity with an oral dose of 240 mg and who had not experienced severe side-effects with this dose were given higher single oral doses (320 and 400 mg) of verapamil on the following days

Also to evaluate the effects of long term oral treatment with verapamil all the patients in group II who exhibited ventricular regularity at any dose level and who did not develop intolerable side effects were given progressively increasing doses of verapamil The dose was increased at 3-day intervals from 80 mg 3 times a day to 240 mg 3 times a day until the patient either showed ventricular regularity

Table II *Cycle length and BP in group I before and after verapamil administration*

RR=mean cycle length (msec) SD=standard deviation of RR interval CV=coefficient of variance of RR intervals
Heart rate and BP were not measured immediately before 80 mg verapamil per os

Case no		Before	After 0 15 mg/kg i v				After 80 mg per os		
			0–2 min	10 min	20 min	30 min	1 h	2 h	3 h
1	RR	998	926	1 043	1 054	1 084	805	973	1 036
	SD	267	129	23	34	20	585	238	225
	CV	26 9	14	2	3	1 8	65	24	21 8
	BP	140/80	120/65	120/60	125/70	130/80	135/75	140/80	150/85
2	RR	905	1 074	1 214	1 033	1 257	848	1 037	1 111
	SD	229	258	190	167	145	227	321	320
	CV	25 4	24	16	14	11 5	27	31	28 8
	BP	120/80	100/65	110/75	105/70	110/70	105/80	120/80	135/85
3	RR	1 063	1 208	1 185	1 212	1 216	1 238	1 299	1 340
	SD	283	445	22	124	5	156	133	118
	CV	26 6	26 6	2	10	0 4	13	10	8 8
	BP	110/80	85/60	115/70	110/70	110/70	110/70	105/65	105/70
4	RR	632	662	779	791	792	647	732	756
	SD	248	171	203	218	215	175	208	219
	CV	39 2	26	26	28	27 2	27	27	28 8
	BP	130/90	120/90	1[illegible]0/80	120/80	125/85	130/85	115/80	110/80
5	RR	615	697	883	882	900	799	907	1 029
	SD	137	165	342	316	291	313	388	417
	CV	22 3	24	39	36	32 3	39	43	40 5
	BP	140/100	140/90	150/90	150/100	130/100	120/75	125/90	130/95
6	RR	842	1 173	1 176	1 374	1 118	1 146	1 112	1 127
	SD	284	516	443	693	310	203	298	249
	CV	33 8	44	38	50	27 8	18	27	22 1
	BP	150/80	130/60	150/75	145/75	150/70	150/70	150/80	150/90
7	RR	979	1 222	1 302	1 327	1 322	1 381	1 393	1 275
	SD	253	301	197	165	181	622	521	514
	CV	25 9	25	15	12	13 6	45	37	40 5
	BP	125/65	110/60	100/60	110/55	105/60	90/60	120/80	110/80
8	RR	796	872	950	976	943	832	965	930
	SD	217	199	173	171	212	254	329	310
	CV	27 4	23	18	17	22 5	31	34	33 2
	BP	125/90	105/75	115/80	120/70	125/85	120/70	110/70	120/80
9	RR	910	1 044	1 242	1 216	1 244	915	1 078	1 112
	SD	249	281	87	115	101	272	274	319
	CV	27 4	27	7	9 5	8 1	30	25 4	29
	BP	110/70	95/60	90/60	100/65	100/70	95/65	100/70	100/65
10	RR	488	657	742	750	779	574	638	598
	SD	136	162	162	156	153	148	135	106
	CV	27 8	25	22	21	19 5	25 5	21 0	17 8
	BP	140/90	125/80	120/80	1[illegible]0/80	120/80	120/80	130/70	120/80
Mean	RR	822	953	1 051	1 061	1 065	918	1 013	1 031
	SD	231 0	262 7	174	215 9	163 3	295 5	284 5	279 7
	CV	28 2	25 8	18 5	20 0	16 4	32 0	29 7	27 1
	BP	129/82	113/70	119/73	123/73	120/76	117/73	121/76	123/81

or had intolerable side-effects of the drug These patients were then evaluated by clinical methods after 4 weeks of therapy

In 5 patients (Table I) ECGs were also recorded during exercise at a load of 20 W for 2 min before drug administration and at the first signs of ventricular regularity as well as after 5–6 hours of drug intake in 4 patients patient 13 could not be exercised at 5 or 6 hours because of marked hypotension

All RR intervals from ECG recordings were calculated and expressed in msec The mean values and standard deviations of all RR intervals from each ECG recording were then calculated In order to assess the regularity of the ventricular response during AF the coefficient of vari

Table III *Cycle length and BP in group II before and after verapamil administration*
Abbreviations as in Table II

Case no		Before	After 240 mg per os 1 h	2 h	3 h	4 h	5 h	6 h	Side effects
11	RR	763	907	985	877	1 110	846	862	Dizziness
	SD	139	170	16	402	269	386	298	bigeminy
	CV	18 2	18 7	1 6	45 8	24 2	45 4	34 5	
	BP	150/80	135/80	110/65	120/70	130/70	130/75	130/75	
12	RR	792	937	1 175	1 242	1 170	996	861	
	SD	234	297	280	254	373	372	200	
	CV	29 6	31 7	23 8	20 5	31 8	37 3	23 1	
	BP	150/90	110/70	110/75	120/80	120/80	125/80	120/80	
13	RR	550	690	750	783	774	611	684	Flushing
	SD	94	63	18	18	101	151	131	of face
	CV	17 2	9 0	2 3	2 3	13 0	22 4	20 2	
	BP	130/90	85/60	80/60	100/70	110/70	120/80	120/80	Hypotension
14	RR	651	866	859	993	1 169	1 233	1 224	Flushing
	SD	151	184	18	23	18	38	64	of face
	CV	23 2	21 2	2 1	2 3	1 7	3 1	5 2	
	BP	150/90	140/90	130/90	130/90	130/90	150/90	150/90	Headache
15	RR	776	927	1 047	1 143	1 128	1 151	1 153	Headache
	SD	143	189	83	126	219	304	256	
	CV	18 0	20 0	8 0	11 0	19 0	26 0	23 0	
	BP	140/80	120/80	120/70	120/65	130/80	130/80	130/80	
16	RR	703	784	992	1 146	1 220	1 149	1 041	
	SD	209	267	422	410	430	366	382	
	CV	29 7	34 0	42 4	35 7	35 2	31 8	36 6	
	BP	170/100	150/90	120/80	120/80	120/80	130/80	130/80	
17	RR	873	841	972	1 118	1 130	1 171	1 214	
	SD	171	18	32	23	32	34	28	Flushing
	CV	19 5	2 0	3 3	3 0	3 0	3 0	2 0	of body
	BP	140/90	120/85	120/85	120/85	120/85	125/85	125/80	
18	RR	870	899	1 121	1 008	991	926	1 075	
	SD	218	246	187	90	49	11	13	
	CV	25 0	27 0	17 0	9 0	5 0	1 0	1 0	
	BP	140/90	130/80	110/75	130/80	130/80	130/80	130/80	
19	RR	504	640	863	839	801	800	721	
	SD	107	163	213	246	204	232	208	Flushing
	CV	21 0	25 0	25 0	29 0	25 0	29 0	29 0	of face
	BP	140/80	130/80	120/80	110/80	110/80	110/80	110/85	
20	RR	536	789	910	845	886	822	698	
	SD	121	173	217	241	204	184	164	
	CV	23 0	22 0	24 0	28 0	23 0	22 0	26 0	
	BP	110/75	110/70	110/70	105/70	100/70	110/75	110/75	
Mean	RR	702	834	967	999	1 032	977	951	
	SD	158 7	177 0	148 6	183 3	189 9	207 8	174 4	
	CV	22 4	21 0	14 9	19 5	18 0	22 1	20 0	
	BP	142/86	123/78	113/75	116/77	120/78	126/80	126/80	

ance was calculated as well A coefficient of variance of 0–12 % was considered to indicate ventricular regularity whereas more than 12 % was interpreted as irregularity of the ventricular response

RESULTS

The effects of i v and oral doses of verapamil on ventricular rate rhythmicity and BP are reported in Tables II and III

Cycle length

The cycle length (RR interval) increased in all patients after i v and oral verapamil This effect was *more pronounced in patients who received* 240 mg by mouth The mean cycle length increased after 240 mg oral verapamil from 702 to 1 032 msec at 4 hours whereas in patients who received 0 15 mg/kg

b wt i v the mean initial RR interval of 822 msec increased to 1065 msec at 30 min after injection Similarly after 80 mg of verapamil by mouth the mean RR increased from 822 to 1031 msec

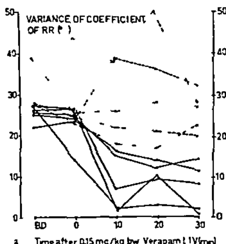
Ventricular rhythmicity

The ventricular response became more regular in 6 patients (nos 11 13 14 15 17 18) who received 240 mg of verapamil by mouth and in 5 (nos 1 2 3 7 9) given i v therapy but only in one patient (no 3) given 80 mg of verapamil by mouth (Fig 1) Fig 2 illustrates examples of ventricular regularity in patients 3 (i v therapy) and 18 (240 mg oral verapamil) Fig 3 shows an actual ECG from a patient before verapamil and with regularizing effect of 240 mg verapamil by mouth

The regularizing effect of 240 mg of verapamil on ventricular rhythmicity was evident between 1 and 3 hours after oral intake of the drug in all 6 patients but this effect persisted for up to 6 hours in only 3 cases Patient 11 developed bigeminy after 3 hours (Fig 1) The 4 patients in group II who did not respond with ventricular regularity to 240 mg of verapamil by mouth did exhibit this effect after 320 mg (2 patients) or 400 mg (2 patients)

Effect of exercise

The effects of exercise on cycle length and ventricular regularity are demonstrated in Table IV Light exercise—a load of 20 W for 2 min—did not alter the ventricular regularizing effect of verapamil Cycle length decreased during exercise both at the first appearance of ventricular regulari



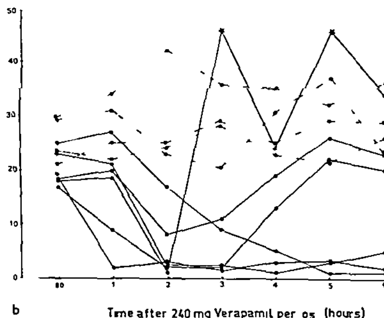
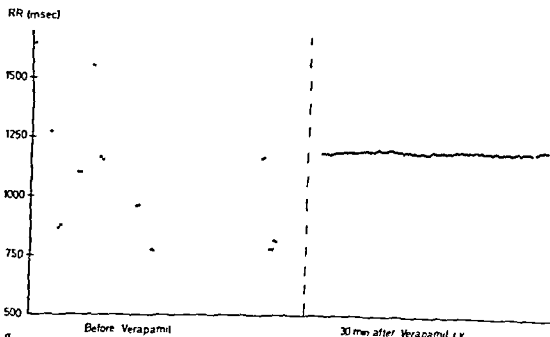


Fig 1 Effect of 0 15 mg verapamil/kg b wt i v (*a*) and 240 mg per os (*b*) on coefficient of variance of RR interval in patients with atrial AF —=Patients who exhibited ventricular regularity =patients who did not ★ the patient who developed bigeminy after verapamil BD=before drug



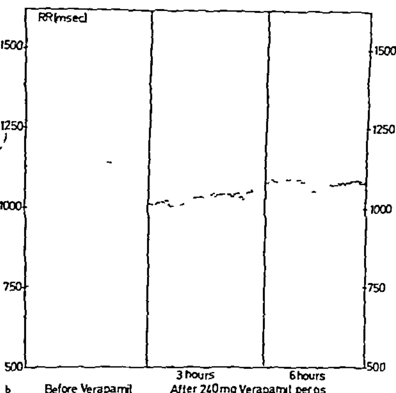


Fig 2 Ventricular regularity after i v (*a*) and oral verapamil (*b*) All consecutive RR intervals recorded during 2 min (i v) and 1 min (240 mg *per os*) have been plotted

ty and 5–6 hours after drug administration except in one patient (no 13) who exhibited a marginal increase in cycle length after exercise at the first appearance of ventricular regularity

Side-effects

A transient drop in systolic BP occurred after verapamil in 18 patients and a marked decrease (below 90 mmHg) in 3 (in two immediately after i v injec

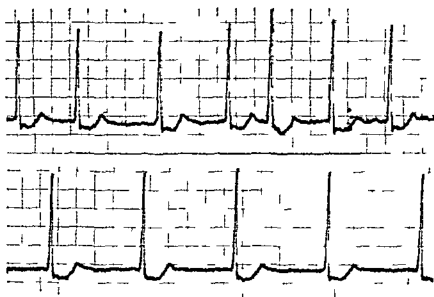


Fig 3 ECG (lead II) from a patient before (*top*) and some hours after 240 mg oral verapamil (*bottom*) The regularizing effect of the drug is obvious

tion and in one 1 hour after oral intake of 240 mg) BP normalized spontaneously in all these patients No other side effects were noted after i v verapamil or oral intake of 80 mg After the higher single oral dose (240 mg) flushing of the face occurred in 4 patients headache in 2 and dizziness in 1 patient These side effects were mild and within the patients tolerance After a dose of 400 mg one patient had severe nausea and flushing of her body

Chronic oral treatment

Chronic oral treatment with verapamil could not be started in 4 patients (nos 14 15 16 18) because of side effects Out of six patients (nos 11 12 13 17 9 20) in whom chronic oral treatment was started three responded with ventricular regularity two at dose level of 240 mg three times a day and one at 60 mg three times a day All three patients had initially a subjective feeling of well being Subsequently however two had to discontinue the treatment because of intolerable side effects (severe nausea and skin rash respectively) the third is still taking 240 mg of verapamil three times a day The other three patients on chronic treatment developed side effects on 80 mg of verapamil three times a day before obtaining any ventricular regularizing effect and the treatment was therefore withdrawn Two exhibited clinical signs of left cardiac decompensation (orthopnoea and basal rales) and one ventricular extrasystoles which disappeared after discontinuation of the drug Both patients who developed decompensation received β blockers in addition to verapamil though one of them not at the time of the oral test but later due to elevated BP

DISCUSSION

It is evident from the results of the present study that none of our patients with AF reverted to SR with either i v or oral verapamil In contrast to this Puech (7) reported reversion to SR in 6 out of 13 verapamil treated patients with AF It is also clear that in our patients low oral doses of verapamil had no appreciable effect on ventricular rhythmicity although they did give a decrease in ventricular rate (increase in cycle length)

The effects of higher oral doses of verapamil on ventricular irregularity were more marked Ventricular regularization occurred in 6 cases which is comparable to the results obtained with i v verapamil (5 cases) in the present series and is also in accordance with the results reported by Schimroth (11) who observed a ventricular regularizing effect of i v verapamil in 15 out of 20 patients with AF Similarly Schamroth et al (12) observed this effect in 71 out of 115 cases of AF

It should be noted that 4 of our patients who did not exhibit ventricular regularization after 240 mg of the drug were given higher single oral doses

Table IV *Effect of exercise studied at the first appearance of ventricular regularity at 1–3 hours after the drug on cycle length (RR) and ventricular regularity in five patients after 240 mg of verapamil per os*
Abbreviations as in Table II

Case no		Before drug		1–3 h after drug (first appearance of ventricular regularity)		5–6 h after drug	
		Resting	Exercise	Resting	Exercise	Resting	Exercise
13	RR	550	476	690	700	–	–
	SD	94	68	63	34	–	–
	CV	17.2	14.1	9.0	4.9	–	–
14	RR	651	603	859	781	1 224	948
	SD	151	124	18	18	64	135
	CV	23.2	20.0	2.1	2.2	5.2	14.0
15	RR	776	621	1 047	662	1 151	673
	SD	143	104	83	86	304	100
	CV	18	17	8	13	26	15
17	RR	873	627	972	806	1 171	743
	SD	171	111	32	19	34	50
	CV	19.5	17.5	3.3	2.3	3	7
18	RR	870	522	1 008	808	926	739
	SD	218	96	90	36	11	17
	CV	25	18	9	4	1	2.3
Mean	RR	744	569	915	751	1 118	775
	SD	155	100	57	38	103	75
	CV	20.5	17.3	6.3	5.2	8.8	9.5

on other occasions. Two patients subsequently showed ventricular regularity after 320 mg and two after 400 mg of verapamil. In another series of 17 patients with AF (5) 240 mg of verapamil by mouth resulted in ventricular regularity in 12. It thus seems possible to induce at least a short period of ventricular regularity during AF in a majority of patients with no contraindications to verapamil treatment. Studies on ventricular regularity over long periods using repeated oral verapamil doses several times daily are required to clarify any benefit of the treatment.

As we did not measure plasma concentrations of verapamil, it is impossible to conclude whether the regularizing effect appears at a certain concentration. Neither can the disparity between the doses needed to achieve this effect be used as a measure of the plasma concentration as verapamil undergoes extensive and individual first pass metabolism (13).

There has been some dispute as to whether the regularization of the ventricular rhythm caused by verapamil in patients with AF is due to complete AV block with nodal escape rhythm or to an unusual pattern of AV conduction (11). Although still regular the ventricular rate can be increased by exercise to a certain extent before irregularity appears again. It is also possible to increase the ventricular rate while still keeping it regular by means of small doses of isoproterenol or atropine (5). These facts might support either of the above mentioned theories about the mechanism of action of verapamil.

Several patients in the present study experienced a feeling of well being during regular rhythm in spite of persistence of AF. This effect may thus be desirable in patients with AF who cannot be converted to permanent SR and who have severe palpitations. However, even patients with AF without subjective discomfort may benefit from the haemodynamic improvement resulting from regular ventricular rhythm (3). We have not evaluated the extent of this improvement, if any, in the present patients.

Verapamil was given to 4 patients who already were on β adrenergic blocking drugs for different reasons. The possible hazard with this combination must be stressed (1, 2, 10). In our experience, however (Olsson, unpublished observations), the oral combination of these drugs in the doses used by us

is tolerated without serious side effects by most patients with a functional deterioration of the de gree in the present series But it is not surprising that the two patients who developed symptoms and s gns of card ac decompensat on had both taken verapamil n addit on to β blockers The poss ble benef ts of the comb ned treatment were however taken into cons deration when the treatment was in t ated It may be assumed that the side-effects due to interference with the contract le mechan sm of the cardiac muscle are dose dependent It is therefore advisable to increase the verapamil dose gradually as we did when planning chron c oral treatment with high doses

In contrast to the verapamil intolerance found in those of our pat ents who were not on β blockers at the same time other workers have found less se vere side effects of verapam l in doses of up to 7?0 mg da ly (6) This dose is tolerated without s de effects in most patients (Puech personal communi cat on) although some may develop flushing as some of our patients did In fact none of the 17 pat ents part cipating in another study and taking ?40 mg of verapam l by mouth had any subjective side effect at all (5)

ACKNOWLEDGEMENT

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REFERENCES

1 Bena m M E Asystole after verapam l Br Med J 2 169 197?

? Boothby C B Garrard C S & Pickering D Vera pam l n card ac arrhythm as Br Med J ? 349 197?

3 G bson D G Broder G & Sowton E Effect of varying pulse nterval n atrial fbrllat on on left ven tricular funct on in man Br Heart J 33 388 1971

4 Jonsell S A method for the determ nat on of the heart s ze by teleroentgenography (A heart volume index) Acta Rad ol (Stockh) ?0 325 1939

5 Khal a A & Olsson S B Verapam l nduced regu lar ventricular rhythm n atrial fbrllat on Stud es w th phys cal exerc se atrop ne and isoproterenol To be publ shed

6 Krikler D Review art cle Verapam l in card ology Eur J Card ol ?/1 3 1974

7 Puech P Experimentat on cl n que de l Isopt ne n jectable et par vo e orale dans le troubles du rythme card aque Centre Hosp talier Montpell er 197?

8 Resnekov L Recent advances n card ology Chur ch ll Liv ngstone Ed nburgh and London 1973

9 Resnekov L & McDonald L Electrovers on of lone atrial fbrllat on and flutter includ ng haemo dynam c stud es at rest and on exercise *Br Heart* J 33 339 1971

10 Sacks H & Kennelly B M Verapam l n card ac arrhythm as Br Med J 2 716 197?

11 Schamroth L Immed ate effects of ntravenous verapam l on atrial fbrllat on Card ovasc Res 5 419 1971

1? Schamroth L Krikler D M & Garrett C Im med ate effects of intravenous verapam l in card ac arrhythm as Br Med J 1 660 1972

13 Schomerus M Sp egelhalde B St eren B & E chelbaum M Phys olog cal d spos t on of vera pam l n man Card o asc Res 10 605 1976

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Effects of Some Cardioactive Drugs on the Oxygen Affinity of Whole Blood

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ABSTRACT Increasing amounts of Digoxin Lasix® Teofyllamin and CI 775 were added to human blood in vitro and the whole blood oxygen affinity was measured We found no definite proof that any of the drugs was able to affect the oxygen affinity of the red cells even at unphysiologically high concentrations

Key words cardioactive drugs oxygen affinity 2 3-diphosphoglycerate plasma pH intraerythrocytic pH

Acta Med Scand 205 49 1979

It has been suggested that the oxygen affinity of blood is increased in patients with severe peripheral arteriosclerosis (3) It is also suggested that the circulating blood of diabetics who have a tendency to develop premature arteriosclerosis has a decreased ability to release oxygen to the tissues (2 9) A decrease in the affinity of hemoglobin for oxygen in these conditions would favour the unloading of oxygen to the tissues Interest has therefore been focussed on drugs and their ability to lower blood oxygen affinity (1 15) It would be theoretically beneficial to lower blood oxygen affinity in patients with angina pectoris or whom the affinity is normal or increased as this should augment oxygen availability to the tissues Propranolol in unphysiologically high concentrations has been shown to decrease blood oxygen affinity in vitro (15) At the therapeutic concentrations encountered in vivo the blood oxygen affinity was not affected (13) According to Corey and Hastings (8) CI 775 which is a cardioselective β adrenergic blocking agent but not propranolol or practolol decreased the oxygen affinity of the blood at therapeutic levels

The aim of this investigation was to examine the effects in vitro of four arbitrarily chosen drugs used in the treatment of cardiovascular diseases namely Digoxin Lasix® Teofyllamin and CI 775 on oxygen affinity of human whole blood

MATERIAL AND METHODS

Drugs

Digoxin 1 ml contains 0 25 mg digoxin 0 1 g spir fortis propylene glycol citric acid natr phosph et aq steril ad 1 ml pH 7 1

Lasix® 1 ml contains 10 mg furosemide natr hydroxide normal et natr chloride q s aq steril ad 1 ml pH 8 9–9 2

Teofyllamin 1 ml contains 23 mg aminophylline (respond 20 mg theophylline 5 5 mg ethylenediamine hydr) natr chloride et aq steril ad 1 ml pH 8 8

CI 775 1 ml contains 10 mg acebutolol et aq steril ad 1 ml pH 5 0–5 8

Blood from 14 healthy non smoking male and female volunteers was used for the experiments Blood samples were drawn twice from 6 volunteers Each drug tested was added to 5 different blood samples and the analyses were performed within 15 min of sampling

Increasing amounts of the drugs—0 001 0 005 0 01 0 02 and 0 04 ml—were added to 1 ml of blood The lowest of these concentrations in the blood was of a magnitude that may occur in vivo using Lasix and Teofyllamin while Digoxin and CI 775 were used in unphysiologically high concentrations In order to obtain baseline values double analyses on blood without any drug added were performed on each volunteer and the mean values of P 50 stand P 50 ery P 50 act pH pl and pH ery were determined (for definitions see below) The technics have been described elsewhere (6) The same parameters were then measured in single analyses on blood to which increasing amounts of each drug had been added The deviation of these parameters from the baseline value of each single individual was then calculated Carboxyhemoglobin (COHb) determined in all

Abbreviations Hb=hemoglobin COHb=carboxyhemoglobin 2,3 DPG=2 3-diphosphoglycerate PO_2=oxygen tension PCO_2=carbon dioxide tension pH pl=plasma pH SO_2=oxygen saturation

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Abbreviations Hb=hemoglobin COHb carboxyhemoglobin 2 3 DPG=2 3 diphosphoglycerate PO_2=oxygen tension PCO_2=carbon dioxide tension pH pl=plasma pH SO_2=oxygen saturation

Table I *Deviation of pH pH ery P50 stand P50 act and P50 ery after addition to whole blood of increasing amounts of Digoxin Lasix Teofyllamin and CI 775 respectively (mean of 5 single analyses in 5 volunteers)*

n=No of analyses performed r=correlation coefficient measuring the change in the above variables with increasing concentrations of the substance p=limit of confidence (10)

ml/ml blood	pH pl	pH ery	P50 stand	P50 act	P50 ery
Digoxin					
0	0	0	0	0	0
0 001	−0 012	+0 016	−0 38	−0 81	−0 24
0 005	−0 026	−0 003	+0 26	−0 61	−0 15
0 01	−0 026	−0 020	+0 85	−0 02	−0 08
0 02	−0 032	−0 023	+0 90	−0 08	0 10
0 04	0 046	−0 037	+3 85	+1 89	+1 86
n	30	30	30	30	30
r	−0 258	−0 332	+0 424	+0 464	−0 187
p	>0 1	>0 05	<0 05	<0 01	>0 1
Lasix					
0	0	0	0	0	0
0 001	−0 012	−0 011	0	−0 25	−0 37
0 005	−0 016	−0 018	−0 42	−0 26	−0 10
0 01	−0 050	−0 012	−1 25	−1 60	−1 48
0 02	−0 026	−0 030	0 58	−1 41	−1 87
0 04	−0 026	−0 023	−0 93	−1 71	−1 76
n	30	30	30	30	30
r	0 189	−0 169	−0 98	−0 191	−0 169
p	>0 1	>0 1	>0 1	>0 1	>0 1
Teofyllamin					
0	0	0	0	0	0
0 001	−0 006	+0 008	+0 05	−0 03	+0 32
0 005	+0 004	+0 010*	−0 69	0 42	−0 24*
0 01	+0 020	0	−1 65	−0 83	−1 47
0 02	+0 032	+0 005	−2 18	−1 18	−1 73
0 04	+0 062	+0 028	−3 26	−1 28	−1 84
	30	29	30	30	29
r	+0 435	+0 176	−0 607	−0 260	−0 329
p	<0 05	>0 1	<0 001	>0 1	>0 05
CI 775					
0	0	0	0	0	0
0 001	−0 002	−0 007	−1 04	−0 90	−0 58
0 005	0	+0 005	−0 88	−0 36	−0 50
0 01	0	+0 014	−0 42	−0 24	+0 24
0 02	0 006	−0 004	+0 23	+1 10	+0 48
0 04	−0 028	−0 006	−0 16	−0 78	+0 60
n	30	30	30	30	30
r	−0 296	0 113	−0 084	−0 038	−0 021
p	0 01	>0 1	>0 1	>0 1	>0 1

* Mean of four observations

samples was normal in all (<1%) except two in which it was slightly increased (1 33 and 1 62%) Analysis of 2 3 diphosphoglycerate (2 3 DPG) was not performed

P50 The P50 value i e the oxygen tension in mmHg required to half saturate Hb was determined on fresh heparinized venous blood drawn in the morning unaerobically from fasting subjects Actual values of oxygen tension (PO_2) carbon dioxide tension (PCO_2) plasma pH (pH pl) oxygen saturation (SO_2) and COHb were determined at 37°C In order to correct pH to 7 40 a Bohr factor −0 48 was used (16) The value of n indicating heme–heme interaction was assumed to be 2 6 (16) and the P50 value was determined by extrapolation using the logarithmic form of Hill s equation (12) Normal values in our laboratory using this technique and at SO_2 values of 42 2–82 3% were 27 1 mmHg±3 6 (2 S D) In the exper

iments this P50 value was designated P50 stand which means that pH pl was used and corrected to pH 7 40 as described above By P50 ery is meant the P50 value obtained using the intraerythrocytic pH (pH ery) This was converted to the corresponding plasma pH value using the formula pH ery=0 743×pH plasma +1 710 as described by Lichtman et al (14) whereafter correction to plasma pH 7 40 was performed By P50 act is meant the P50 value obtained at the actual plasma pH without correction to 7 40

Intraerythrocytic pH Two capillary tubes with an inner diameter of 2 5 mm were used for each measurement One end was closed with putty the tube was filled with blood and centrifuged at 1 100 *g* for 10 min (occluded end down) A clear hemolysate was obtained after repeated thawing and freezing The part of the tube containing plasma was sawn off and the pH of the hemolysate was determined

Facilities for laboratory investigations were offered by Professor S E Lindell Department of Clinical Physiology Malmo General Hospital

RESULTS

When increasing amounts of Digoxin were added to the blood there was an increase in P50 stand and in P50 act P50 ery pH pl and pH ery were not significantly affected There were no significant changes when Lasix or CI 775 were added to the blood With increasing amounts of Teofyllamin there was a significant increase in pH pl and a significant decrease in P50 stand The other parameters were not affected (Table I)

DISCUSSION

The oxygen affinity for Hb is affected by the four physiological ligands i e protons CO_2 2 3 DPG and CO pH is usually measured in plasma and it is assumed that a change in plasma pH is accompanied by a concomitant change in intraerythrocytic pH However when determining the oxygen affinity the intraerythrocytic pH should be taken into account This was shown to be essential when radiographic contrast media were used impenetrant compounds did not affect the oxygen affinity when the intraerythrocytic pH was taken into consideration (6 14) In our experiments 2 3 DPG was not determined but it should be emphasized that the analyses were performed within 15 min of drawing the blood samples Analytical methods usually stipulate that the blood should be diluted for assay within 5 min or stored in an ice bath for no more than 2 hours (4 11) However according to Hellerstein and Bunthrarungroj (11) there were no significant alterations in P50 or 2 3 DPG when blood was permitted to stand before analysis at room temperature for periods of 1–7 hours Carbon monoxide was determined in all samples and was normal in all (<1%) except two in which it was slightly increased (1 33 and 1 62%)

Modulation of oxygen affinity by pharmacological means might be useful in several diseases In an attempt to identify agents which might improve oxygen release from Hb we examined the effects in vitro on whole blood oxygen affinity after addition of four arbitrarily chosen drugs The ability of propranolol to reduce oxygen affinity of Hb in human red cells in vitro has been described by several authors although not at the therapeutic levels encountered in vivo (1 13 15) Corey and Hastings (8) found CI 775 to decrease the oxygen affinity of blood at therapeutic levels Our experiments did not confirm this finding as no changes were observed when CI 775 was added to the blood We are unable to explain these conflicting results Nor was the oxygen affinity affected when increasing amounts of Lasix were added to the blood On the other hand the addition of Teofyllamin caused a decrease in P50 stand which could be explained by an increase in plasma pH The intraerythrocytic pH and P50 ery however were not affected It remains unclear why the addition of Lasix of the same alkaline pH as that of Teofyllamin did not produce any change in plasma pH The increases in P50 stand and P50 act upon the addition of Digoxin also remain unclear as the plasma pH remained unaffected The cause may have been methodological as the scatter was considerable in the P50 stand experiments although less pronounced in the P50 act determinations Most important pH ery and P50 ery were not affected

In conclusion we found no definite proof that any of the drugs tested was able to affect the oxygen affinity of the red cells Other drugs may be effective in this respect Theoretically lowering the oxygen affinity of Hb may be of value in patients on the brink of disaster with severe angina pectoris Here an increase in the unloading of oxygen to the tissues may be beneficial It is however worth noting that people with abnormal Hb so avid for oxygen as to cause tissue hypoxia and secondary erythrocytosis e g Hb Chesapeake (7) and Hb Malmo (5) apparently cope very well with their inborn anomaly

REFERENCES

1 Agostoni A Berfasconi C Gerli G C Luzzana M & Rossi Bernardi L Oxygen affinity and electrolyte distribution of human blood Changes induced by propranolol Science 182 300 1973

2 Artursson G Garby L Robert M & Zaar B Oxygen affinity of whole blood in vivo under standard conditions in subjects with diabetes mellitus Scand J Clin Lab Invest 34 19 1974

3 Astrup P Oxygen dissociation curves in some diseases Forsvarsmedicin 5 199 1969

4 Atkinson K F Modified automated determination of 2 3 DPG in whole blood Clin Chem 20 649 1974

5 Berglund S Erythrocytosis associated with haemoglobin Malmö accompanied by pulmonary changes occurring in the same family Scand J Haematol 9 355 1972

6 Berglund S Almen T & Johansson B W Effect of Metrizamide on whole blood oxygen affinity Acta Radiol In press 1978

7 Charache S Weatherall D J & Clegg J B Polycythemia associated with a hemoglobinopathy J Clin Invest 45 813 1966

8 Corey S & Hastings S G The effects of CI 775 on the oxyhemoglobin dissociation curve of dog and human blood Parke Davis internal report to D A McCarthy October 20 1975

9 Ditzel J & Standl E The problem of tissue oxygenation in diabetes mellitus Acta Med Scand (Suppl) 578 49 1975

10 Documenta Geigy Wissenschaftliche Tabellen 6th ed 170 (5) 1960

11 Hellerstein S & Bunthrarungroj T Effects of time and temperature on blood P50 and 2 3 DPG measurements Clin Chem 22 (1) 39 1976

12 Hill A V The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves J Physiol (Lond) 40 4 1966

13 Lichtman M A Cohen J Murphy M S Kearney E A & Whitbeck A A Effects of propranolol on oxygen binding to hemoglobin in vitro and in vivo Circulation 49 881 1974

14 Lichtman M A Whitbeck A A & Murphy M Factitious changes in binding of oxygen to hemoglobin when based on extracellular pH in the presence of certain blood additives like radiographic contrast media Invest Radiol 10 225 1975

15 Pendelton R G Newman D J Sherman S S Brann E G & Maya W E Effects of propranolol upon the hemoglobin–oxygen dissociation curve J Pharmacol Exp Ther 180 647 1972

16 Severinghaus J Blood gas calculator J Appl Physiol 21 1108 1966

Evaluation of Quinidine Lipettes® — a Sustained Release Preparation

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ABSTRACT A new sustained release preparation (Lipettes®) of quinidine has been evaluated with regard to dissolution absorption serum concentration and side-effects The serum levels of quinidine after single oral doses and after long term treatment have been compared with the serum levels after administration of some other quinidine preparations on the Swedish market The side-effects of the sustained release preparations have also been studied Results indicated that this new sustained release quinidine preparation yields more even serum concentrations of quinidine and seemed to cause less troublesome side-effects than the marketed preparations

Key words quinidine plasma concentration
Acta Med Scand 205 53 1979

Quinidine is one of the most frequently used antiarrhythmic agents It is almost completely absorbed after oral administration The absorption takes place mainly in the proximal part of jejunum (17) but also in the more distal parts of the intestine and in rectum (10 16) Drugs with this kind of absorption pattern can be used in sustained release preparations and still be adequately available

The correlation between the serum levels of the alkaloid and the therapeutic effect is good The commonly accepted therapeutic range of quinidine in serum determined according to the method of Cramer and Isaksson (4) is 1–4 mg/l (3–12 μmol/l) The serum levels of quinidine may differ widely between individuals after equal doses of the same preparation but in each individual the conditions seem to be fairly constant (5 19)

The most common side effects of quinidine therapy within the therapeutic serum range of quinidine are gastrointestinal symptoms such as nausea vomiting and diarrhea probably due to local irritation of the gastrointestinal tract Also neurological side-effects such as tinnitus blurred vision headache and vertigo may occur at this serum level (3 15) Repeated high peaks and marked fluctuations in serum concentration of quinidine can probably influence the frequency of side effects (5 6 8)

In the present investigation a new release preparation (Lipettes®) of quinidine has been evaluated This preparation was expected to give a lower initial absorption peak of quinidine more even serum levels and thereby a decreased frequency of side effects compared to available preparations

EXPERIMENTAL

Preparations

Quinidine Lipettes® are manufactured according to a moulding principle (20) The active component is suspended or dissolved in a melt of hydrophobic and hydrophilic substances By changing the proportions of the hydrophobic and hydrophilic components the dissolution rate of the active ingredient can be varied The dissolution of the active ingredient occurs by erosion from the surface and by release from the core No solid residues will be found in feces

Three preparations of Quinidine Lipettes (A B and C) containing 0 2 g quinidine sulphate were manufactured The proportion of hydrophobic and hydrophilic components in preparation A B and C were 1 2 3 2 and 4 1 respectively The Lipettes were coated with a suspension based on Macrogol 6000 which yields a film that is easily dissolved in gastrointestinal fluids

Quinidine Durules® have been marketed in Sweden for several years They contain quinidine bisulphate in a quantity equivalent to 0 2 g quinidine sulphate Durules® are of the plastic matrix type with sustained release properties The dissolution of the drug from the Durules takes place by physical diffusion the rate of which depends on solubility of the drug properties of the vehicle and porosity of the tablet

Cardioquin® tablets contain quinidine polygalacturonate in a quantity equivalent to 0 2 g quinidine sulphate The tablets are of a conventional type with rapid disintegration However the in vitro dissolution rate is dependent on the pH of the dissolution media In simulated

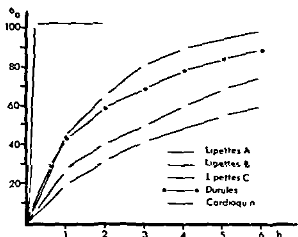


Fig 1 Dissolution rate of quinidine in simulated gastric fluid from five different preparations

gastric and intestinal fluids dissolution is rapid while it is very slow in water

Analytical methods Serum quinidine concentrations were determined according to Cramer and Isaksson (4)

Statistical methods The statistical calculations were performed according to Fisher (7)

Screening procedures

Dissolution test The dissolution tests in vitro of the quinidine preparations were performed in simulated gastric fluid without enzymes at 37°C with a rotation basket apparatus according to U S P XIX at a speed of 100 rpm (13) The results of the dissolution tests are shown in Fig 1 As expected the dissolution rate of quinidine from the three Lipettes increased with increasing proportions of the hydrophilic component The dissolution rates of Durules and Lipettes A were rather similar From the Cardioquin tablets the quinidine dissolution was very rapid

Absorption screening of Lipettes The absorption pattern of quinidine from Lipettes A B and C and from Durules were studied in a cross-over design in six hospitalized patients in the postmyocardial infarction stage All were male with a mean age of 58 years (range 51–65) Serum levels of quinidine after administration of a single dose of 0 1 g quinidine sulphate/10 kg b wt of the different preparations are shown in Fig 2 Lipettes A gave the highest absorption peaks and serum levels while Lipettes C gave the lowest values Thus the in vivo results showed a fair correlation to the in vitro results

From this absorption study Lipettes B was considered the most suitable preparation to meet the requirements i e to yield as constant serum levels of quinidine as possible without pronounced absorption peaks

Short term clinical studies

Serum concentration of quinidine after single doses of Lipettes B and Durules After the screening absorption test the serum levels during a 24 hour period were further investigated after administration of Lipettes B and Durules Nine persons participated after informed consent and they all received 0 1 g quinidine sulphate/10 kg b wt of the two preparations at 3-day intervals in a cross-over design All were male hospitalized patients in the postmyocardial stage mean age 58 years (range 51–72) Blood samples for quinidine analysis according to Cramer and Isaksson (4) were drawn immediately before and 1 2 3 4 5 8 12 and 24 hours after administration of the tablets which were taken half an hour before breakfast The meals during the test days were of similar composition and were always taken at 8 a m noon and 5 p m The individual and mean serum concentrations of quinidine in the nine persons during 24 hours after oral administration of Lipettes B and Durules are shown in Figs 3 4 and 5

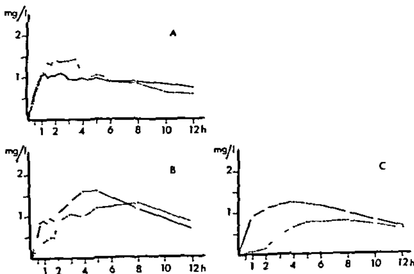


Fig 2 Serum concentrations of quinidine after oral administration to six patients of three Quinidine Lipettes preparations (A B and C) (broken lines) and Quinidine Durules (solid line)

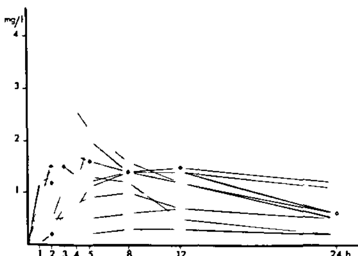


Fig. 3. Serum concentrations of quinidine in nine postinfarction patients after oral administration of Quinidine Lipettes B in a dosage of 0.1 g quinidine sulphate/10 kg b.wt.

As shown in Figs. 3 and 4, the maximum concentration of quinidine was reached 4.6±1.0 hours and 3.7±0.3 hours respectively after administration and was 1.4±0.2 mg/l (4.3±0.6 µmol/l) for Lipettes B and 2.1±0.3 mg/l (6.5±0.9 µmol/l) for Durules. The Lipettes yielded significantly lower serum quinidine levels during the first 5 hours (p 0.05–0.01, t test for intra-pair differences between the preparations), but there was no significant difference after 8, 12 and 24 hours (p 0.6–0.05). Furthermore, as shown in Table I, serum levels above 90, 80, 70 and 60% of the maximum concentrations of quinidine in serum were maintained for longer by Lipettes than by Durules.

Analysis of variance was applied to the serum concentration values obtained between 3 and 12 hours after administration of the two preparations. Lipettes did not give any statistically significant difference between the hours ($F<1$, d.f. 32/4, $p>0.05$), unlike Durules (F 16.0, d.f.=32/4, $p<0.001$). This indicates a more even serum concentration of quinidine after administration of Lipettes than after administration of Durules.

Serum concentration of quinidine after multiple doses of Lipettes B and Durules. The study was carried out in order to investigate whether Lipettes gave more even serum concentrations than Durules also under steady state conditions. Most of the patients were receiving additional therapy with digitalis and diuretics and had been in a steady state as regards these drugs for at least two weeks. Five postinfarction male patients participated, after informed consent, and started the medication with Lipettes or Durules in a randomized order, the therapy with each preparation being maintained for 7 days. The patients who had received Lipettes first were then given Durules and vice versa for another period of 5 days. During the last day of medication with the respective preparation the serum levels of quinidine were followed during 12 hours in 4 of the patients (2, 4, 6, 8 and 12 hours after the morning dose at 8 a.m.). The daily doses of quinidine, which were the

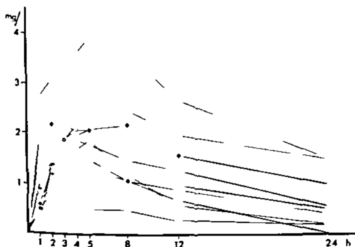


Fig. 4. Serum concentrations of quinidine in nine postinfarction patients after oral administration of Quinidine Durules in a dosage of 0.1 g quinidine sulphate/10 kg b.wt.

Table I *Difference in hours during which the serum concentration exceeded 90 80 70 and 60% of the maximum concentration of quinidine in serum after equivalent single doses of Lipettes and Durules*

	Difference in hours Lipettes–Durules at			
	90%	80%	70%	60%
Mean of difference (h)	2 4	6 2	8 5	9 7
$\pm$ S E M $_{diff}$	1 3	2 5	3 7	4 1
t	1 846	2 490	2 297	2 366
d f	8	8	8	8
p	0 05<p<0 1	0 02<p<0 05	0 05<p<0 1	0 02<p<0 05
Confidence interval at p=0 05	2 4±3 0	6 2±5 8	8 5±8 6	9 7±9 5

same for Lipettes and Durules were adjusted to the need of each patient and were about 0 1 g quinidine sulphate/10 kg b wt The tablets were administered in equal doses at 8 a m and 8 p m

Fig 6 shows the morning concentration of quinidine in the 3 patients who started with Lipettes As can be seen *the steady state level was reached after 3–4 days of medication* and was the same after the change to Durules Difference in mean steady state levels 0 15±0 18 mg/l (0 46±0 55 μmol/l t=0 833 p>0 4)

Fig 7 shows the serum concentrations of quinidine during the last day of treatment with Lipettes and Durules respectively The results are from four patients since one *had left the hospital before the study was completed* Analysis of variance applied to the serum concentration values obtained between the morning dose at 8 a m and the evening dose at 8 p m showed that Lipettes did not give statistically significant differences between the hours (F=2 750 d f =5/14 p>0 05) whereas Durules did (F=6 429 d f =5/15 p<0 01)

This indicates that the serum levels after administration of Lipettes are more even than after Durules also under steady state conditions

Long term clinical studies

Morning concentrations of quinidine in serum and side effects during long term treatment with Lipettes Durules and Cardioquin Thirty-eight patients (31 male and 7 female mean age 59 years range 39–76) from the Myocardial Infarction and the Out Patient Clinics participated in the study which was carried out in a cross over design All patients received Lipettes and Durules for periods of 3–4 weeks 31 also received Cardioquin during a *third period of the same length* The daily dosage was adjusted individually and was usually 0 8 g quinidine sulphate for patients weighing less than 70 kg and 1 2 g for patients weighin more than 70 kg The individual dosages of quinidine were the same throughout the test periods and were administered twice a day in equal doses at 8 a m and 8 p m

At the end of each treatment period the concentrations of quinidine in serum were determined at 8 a m before the morning dose had been administered and side-effects caused by the treatment were recorded through a standardized questionnaire After the long term treatment there was no significant difference in the serum concentrations of quinidine at 8 a m —before administration of the morning dose—between the three preparations

Table II shows the total daily intake of quinidine and the mean serum concentrations in patients from whom blood samples for quinidine analysis were drawn after 3–4 weeks treatment

The side-effects caused by Lipettes Durules and Cardioquin were mainly diarrhea and other gastrointesti

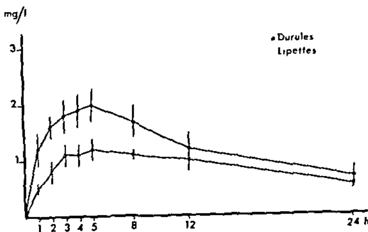


Fig 5 Serum concentrations of quinidine in nine patients after oral administration of Quinidine Lipettes B and Quinidine Durules (mean ± S E M)

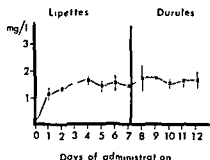


Fig. 6. Morning serum concentrations of quinidine in 3 patients during 13 days at 8 a.m. after oral administration of Quinidine Lipettes and Quinidine Durules (mean ± S.E.M.).

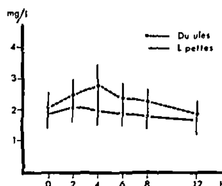


Fig. 7. Serum concentration of quinidine in 4 patients during 12 hours under steady state condition after oral administration of Quinidine Lipettes and Quinidine Durules (mean ± S.E.M.).

nal symptoms. Central side-effects such as nausea and dizziness also occurred. Furthermore the gastrointestinal side-effects seemed to be more frequent during the initial phase of the quinidine administration. Since none of the patients received Cardioquin as the first or second preparation and the side-effects showed a tendency to decrease during quinidine treatment, the side-effects of Cardioquin have been compared with those of Lipettes and Durules given during the second 3–4 week period of quinidine administration.

Lipettes gave gastrointestinal side-effects during the first treatment period with quinidine in 63.6% of the patients and Durules in 44.4%. This difference is not significant ($p<0.1$). When Lipettes were given during the second 3–4-week period after Durules, the frequency of side-effects was reduced to 25.9%. On the other hand when Durules were given after Lipettes, the frequency of side effects was increased to 63.6%. This reduction of the gastrointestinal side-effects during the subsequent treatment with Lipettes is statistically significant ($\chi^2=4.777$, d.f.=1, $p<0.05$).

Cardioquin induced gastrointestinal side-effects in 45.2% of the patients in the third 3–4 week period, while the two other preparations together gave gastrointestinal side-effects in 35.5% of the patients during the second period. This increase in the frequency of side-effects of Cardioquin is not statistically significant ($\chi^2=0.603$, d.f. 1, $p>0.1$).

Five patients experienced nausea and/or dizziness during treatment with Durules and one of them had the same symptoms while on treatment with Lipettes. As can be seen in Table III, there was no significant difference in the morning serum concentration in the 5 patients who complained of central side-effects irrespective of preparation.

DISCUSSION

Sustained release preparations of quinidine have been introduced to overcome the high initial serum peaks, as well as the fluctuations in serum levels, and to reduce the number of administrations.

In this investigation a new sustained release preparation (Lipettes) of quinidine was compared with one already available (Quinidine Durules) in order to evaluate whether the new preparation would

Table II. *Morning serum concentration of quinidine after 3–4 weeks' treatment with Lipettes, Durules and Cardioquin*

	Lipettes	Durules	Lipettes	Cardioquin	Durules	Cardioquin
No. of pats	36		26		24	
Intake of quinidine sulphate (g/24 h) ± S.E.M.	1.02±0.06		1.06±0.08		1.04±0.10	
Morning concentration of quinidine (mg/l) ± S.E.M.	1.9±0.1	2.1±0.1	2.0±0.1	2.1±0.2	2.2±0.2	2.1±0.2
Morning serum concentration range (mg/l)	0.5–2.9	0.6–4.3	0.5–2.9	0.6–3.9	0.6–4.3	0.6–3.9
Difference in serum concentration (mg/l) ± S.E.M.	−0.14±0.08		−0.13±0.01		0.05±0.11	
t	1.750		1.300		0.457	
d.f.	35		25		23	
p	>0.05		>0.1		>0.6	

Table I *Difference in hours during which the serum concentration exceeded 90 80 70 and 60% of the maximum concentration of quinidine in serum after equivalent single doses of Lipettes and Durules*

	Difference in hours Lipettes–Durules at			
	90%	80%	70%	60%
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p	$0\ 05<p<0\ 1$	$0\ 02<p<0\ 05$	$0\ 05<p<0\ 1$	$0\ 02<p<0\ 05$
Confidence interval at $p=0\ 05$	2 4±3 0	6 2±5 8	8 5±8 6	9 7±9 5

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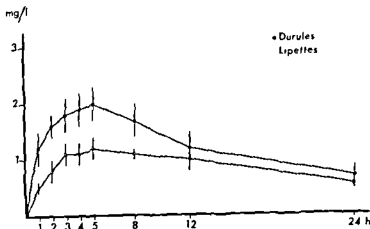


Fig 5 Serum concentrations of quinidine in nine patients after oral administration of Quinidine Lipettes B and Quinidine Durules (mean ± S E M)

Table I *Changes in BP after treatment with atenolol alone or in combination with other drugs*
S=supine E=erect

	No previous treatment			Previous treatment with other β-blockers			Previous treatment without β-blockers		
Present treatment	*n*	Mean dosage (mg)	ΔBP (mmHg) Syst./diast.	*n*	Mean dosage (mg)	Further ΔBP (mmHg) Syst./diast.	*n*	Mean dosage (mg)	Further ΔBP (mmHg) Syst./diast.
Tenormin	11	81	−38 6/20 1 S −39 4/18.3 E	7	121	−16 2/8.3 S −14.5/7 2 E	0	–	–
Tenormin+vasodilator (hydralazine or prazosin)	4	175	−37 0/19.2 S −32 9/29 1 E	11	238	−10.3/6.3 S −8 3/5 8 E	0	–	–
Tenormin+vasodilator+spironolactone	1	300	−16 7/20 0 S −16 7/10 0 E	8	200	−21 0/13 8 S −14 8/16 7 E	1	100	−45 0/35 0 S −36 3/21 7 E
Tenormin+diuretics (thiazides)	3	143	−17 8/10 0 S −11 2/7.3 E	9	147	−16 8/12 8 S −11 0 6 7 E	2	125	−17.5/14 2 S −25 8/23 2 E
Tenormin+vasodilator+diuretics (thiazides)	2	200	−59 2/15 8 S −69.2/24 2 E	8	221	−32 4/15 0 S −30.5/21 4 E	0	–	–
Tenormin+spironolactone	3	168	−42.3/23 9 S −38 9/18.3 E	4	156	−43.3/30.5 S −37.5/23 7 E	4	94	−22.1/20 4 S −8 0/12 6 E
Tenormin+various (bethanidine methyldopa clonidine)	0	–	–	6	183	−16 1/7 3 S −22 2/12 8 E	2	75	−10 0/20 0 S −21 5/20 6 E
	24			53			9		

eliminated with urine and 50% with feces Only three to four metabolites are known and they have no significant biological activity (4)

Patients

far 104 patients have been recruited for treatment atenolol Thirty six are women with an average age 54 years (range 31–63) and 68 men with an average age of 50 years (range 26–65) The diagnoses were essential or cryptogenic hypertension in 97 (93%) renal hypertension in 4 (4%) and renovascular hypertension in 3 (3%)

According to the WHO classification 56 (54%) patients belong to group I 26 (25%) to group II and 22 (21%) to group III In 15 patients treatment with atenolol was decided because of a co-existing chronic obstructive pulmonary disease or a positive history of asthmatic attacks In all these cases extensive lung-physiological investigations were made before and several times during the treatment. A more specific evaluation of these results will be published elsewhere (13)

Design of the study

The investigation was designed as an open study and all patients were treated in the Hypertension Unit. All BPs were taken by nurses—mostly the same nurse All patients were controlled before noon but in most cases afternoon or evening controls were made as well In the group of patients with mild hypertension (WHO I) at least three BPs were taken before initiation of treatment The whole scale of metabolic parameters was covered Hb leucocytes sodium potassium calcium creatinine uric acid fasting blood glucose cholesterol triglycerides and 12 lead ECG Urine was tested for glucose protein and sediments On the slightest suspicion of secondary hypertension further investigations were performed e g i v urography (a m Maxwell) isotope renographs and/or selective renal angiography

All BPs were measured indirectly with a mercury manometer after 5–10 min recumbency and 1–2 min standing. Disappearance of the Korotkoff sounds (phase V) was taken as the diastolic BP Controls were made every fortnight at the beginning of treatment but after the target pressure (diastolic BP≤95 mmHg) had been reached or the diastolic BP had dropped more than 10% the patients were controlled at longer intervals

RESULTS

A total of 104 patients were treated for 8–36 months with 12 5–300 mg atenolol per day Eighteen patients discontinued treatment for various reasons—see below under Side-effects The mean duration of treatment was 16 months which is the longest follow up time we are aware of (10)

In the whole series 62 patients had had previous treatment—a sub t part of whom were well controlled—and t s instituted because of suspected or cl see below

6 Cullhed I Hamfelt A & Malers E Serum quinidine concentration with two long acting quinidine preparations Acta Med Scand 179 401 1966

7 Fisher R A Statistical methods for research workers 12th ed Oliver & Boyd Edinburgh 1954

8 Hartel G Louhija A Konttinen A & Halonen P I Value of quinidine in maintenance of sinus rhythm after electric conversion of atrial fibrillation Br Heart J 32 57 1970

9 Henning R & Nyberg G Serum quinidine levels after administration of three different quinidine preparations Eur J Clin Pharmacol 6 239 1973

10 Kalmansohn R W & Sampson J J Studies of plasma quinidine content I Relation to single dose administration by three routes Circulation 1 564 1950

11 Lindseth Ditlefsen E M Concentrations of quinidine in blood following delayed absorption tablets Acta Med Scand 149 49 1954

12 Lindseth Ditlefsen E M & Bjerkelund C Quinidine concentration in serum The stability during maintained treatment with two different types of delayed absorption tablets Acta Med Scand 180 537 1966

13 Pharmacopeia of the United States of America ed XIX United States Pharmacopeial Convention Inc Rockville 1975

14 Renais L S & Lenegre J Etude des taux quinidinemie obtenus par l'administration d'une quinidine retard Arch Mal Cœur 64 701 1971

15 Resnekov L Gibson D Waich D Muir J & McDonald L Sustained release quinidine (Kinidin Durules) in maintaining sinus rhythm after electroversion of atrial dysrhythmias Br Heart J 33 220 1971

16 Sampson J J Foreman H & Solomon B C Studies of plasma quinidine content III The value of delayed absorptive coated tablets in oral quinidine therapy Circulation 5 534 1952

17 Shanker L S Tocco D J Brodie B B & Hogben C A M Absorption of drugs from the rat small intestine J Pharmacol Exp Ther 123 81 1958

18 Sokolow E & Edgar A L Blood quinidine concentrations as a guide in treatment of cardiac arrhythmias Circulation I 576 1950

19 Sokolow M & Ball R E Factors influencing conversion of chronic atrial fibrillation with special reference to serum quinidine concentration Circulation 14 568 1956

20 Wahlgren S Lipetter – en tablettvariant med utvecklingsmojligheter Svensk Farm Tidskr 74 207 1970

Table I *Changes in BP after treatment with atenolol alone or in combination with other drugs*
S=supine E=erect

Present treatment	No previous treatment			Previous treatment with other β blockers			Previous treatment without β blockers		
	n	Mean dosage (*mg*)	ΔBP (mmHg) Syst /*diast*	*n*	Mean dosage (*mg*)	Further ΔBP (mmHg) Syst /*diast*	*n*	Mean dosage (mg)	Further ΔBP (mmHg) Syst /diast
Tenormin	11	81	−38 6/20 1 S −39 4/18 3 E	7	121	−16 2/8 3 S −14 5/7 2 E	0	–	–
Tenormin+vasodilator (hydralazine or prazosin)	4	175	−37 0/19 2 S −32 9/29 1 E	11	238	−10 3/6 3 S −8 3/5 8 E	0	–	–
Tenormin+vasodilator+ spironolactone	1	300	−16 7/20 0 S −16 7/10 0 E	8	200	−21 0/13 8 S −14 8/16 7 E	1	100	−45 0/35 0 S −36 3/21 7 E
Tenormin+diuretics (thiazides)	3	143	−17 8/10 0 S −11 2/7 3 E	9	147	−16 8/12 8 S −11 0/6 7 E	2	125	−17 5/14 2 S −25 8/23 2 E
Tenormin+vasodilator+ diuretics (thiazides)	2	200	−59 2/15 8 S −69 2/24 2 E	8	221	−32 4/15 0 S −30 5/21 4 E	0	–	–
Tenormin+spironolactone	3	168	−42 3/23 9 S −38 9/18 3 E	4	156	−43 3/30 5 S −37 5/23 7 E	4	94	−22 1/20 4 S −8 0/12 6 E
Tenormin+various (*bethanidine methyldopa* clonidine)	0	–	–	6	183	−16 1/7 3 S −22 2/12 8 E	2	75	−10 0/20 0 S −21 5/20 6 E
	24			53			9		

eliminated with urine and 50% with feces Only three to four metabolites are known and they have no significant biological activity (4)

Patients

far 104 patients have been recruited for treatment ı atenolol *Thirty six are women with an average age* ¸4 years (range 31–63) and 68 men with an average age of 50 years (range 26–65) The diagnoses were essential or cryptogenic hypertension in 97 (93%) renal hypertension in 4 (4%) and renovascular hypertension in 3 (3%)

According to the WHO classification 56 (54%) patients belong to group I 26 (25%) to group II and 22 (21%) to group III In 15 patients treatment with atenolol was *decided because of a co-existing chronic obstructive* pulmonary disease or a positive history of asthmatic attacks In all these cases extensive lung physiological investigations were made before and several times during the treatment A more specific evaluation of these results will be published elsewhere (13)

Design of the study

The investigation was designed as an open study and all patients were treated in the Hypertension Unit All BPs were taken by nurses—mostly the same nurse All patients were controlled *before noon but in most* cases afternoon or evening controls were made as well In the group of patients with mild hypertension (WHO I) at least three BPs were taken before initiation of treatment The whole scale of metabolic parameters was covered Hb leucocytes sodium potassium calcium creatinine uric acid fasting blood glucose cholesterol triglycerides and 12 lead ECG Urine was tested for glucose protein and sediments On the slightest suspicion *of secondary hypertension further investigations were* performed e g i v urography (a m Maxwell) isotope *renography and/or selective renal angiography*

All BPs were measured indirectly with a mercury manometer after 5–10 min recumbency and 1–2 min standing Disappearance of the Korotkoff sounds (phase V) was taken as the diastolic BP Controls were made every fortnight at the beginning of treatment but after the target pressure (diastolic BP≤95 mmHg) had been reached or the diastolic BP had dropped more than 10% the patients were controlled at longer intervals

RESULTS

A total of 104 patients were treated for 8–36 months with 12 5–300 mg atenolol per day Eighteen patients discontinued treatment for various reasons—see below under Side effects The mean duration of treatment was 16 months which is the longest follow up time we are aware of (10)

In the whole series 62 patients had had previous treatment—a substantial part of whom were well controlled—and the change was instituted because of suspected or clear side effects (see below) The

disease could be found and the biopsies showed only a non specific toxic dermatitis

In a total of 10 patients we registered disappearance of symptoms after cessation of atenolol in five of them the side effects were quite non specific and more related to the β blockade i e bradycardia cold extremities and muscle fatigue

DISCUSSION

Atenolol has in several studies shown its usefulness in the treatment of hypertension and this is in line with experience from other β blockers Atenolol seems to lack side effects from the CNS when given in the normal dosage range and therefore seems to have an advantage over the more lipid soluble and/or non selective β blockers Zacharias et al (26) have described many side-effects during therapy with propranolol and about 10% of their patients complained of suspected CNS side effects but treatment was discontinued in only a few cases We have found that about 20–25% of all propranolol treated patients sooner or later develop some CNS related side-effects and quite many of them begin to complain of these after several months of treatment—in five cases in this material after at least one year s treatment with propranolol We also believe that the patient s compliance for attenuation of therapy and control of BP is very much based on the lack of side-effects during the therapy Very few studies have included a comparison between various β blockers in respect to their CNS side-effects (7)

Propranolol has a significant effect on migraine and so it seems has pindolol (2 18) Two of our patients had been treated with success for migraine and hypertension with either propranolol or pindolol but their symptoms returned when they started treatment with atenolol Both patients preferred to continue with atenolol but one female continued also with 5 mg pindolol daily with good relief of her symptoms It may be that atenolol has little or no effect on migraine whereas other non selective more CNS penetrating β blockers (propranolol alprenolol pindolol oxprenolol) and even selective but also CNS penetrating β blockers (metroprolol) give satisfactory results in this common disease

One female became pregnant and atenolol was withdrawn after two months of pregnancy She later gave birth to a completely normal boy now 18 months old

REFERENCES

1 Åstrom H & Vallin H Effect of a new beta adrenergic blocking agent ICI 66 082 on exercise hemodynamics and airway resistance in angina pectoris Br Heart J 36 1194 1974
2 Börgesen S E Nielsen J L & Moller C E Prophylactic treatment of migraine with propranolol Acta Neurol Scand 50 651 1974
3 Case D E Personal communication
4 Conway F J Fitzgerald J D McAinsh J Rowlands D J & Simpson W T Human pharmacokinetic and pharmacodynamic studies on atenolol (ICI 66 082) a new cardioselective beta adrenoceptor blocking drug Br J Clin Pharmacol 3 267 1976
5 Felix R H Ive F A & Dahl M G C Cutaneous and ocular reactions to practolol Br Med J IV 321 1974
6 Formgren H Practolol in the treatment of tachyarrhythmias in patients with bronchial asthma Am Heart J 84 710 1972
7 Hansson L Beta adrenergic blockade in essential hypertension Effects of propranolol on hemodynamic parameters and plasma renin activity Acta Med Scand (Suppl) 550 1 1973
8 Hansson L Henningsen N C Karlberg B E Åberg H Jameson S Malmcrona R & Hersvall C Hypotensive action of ICI 66 082 a new beta adrenergic blocking agent Int J Clin Pharmacol 10 206 1974
9 Hansson L Karlberg B E Åberg H Westerlund A Henningsen N C & Jameson S Clinical evaluation of atenolol in hypertension Clin Sci Mol Med 51 513 1976
10 Hansson L Karlberg B E Åberg H Westerlund A Jameson S & Henningsen N C Long term hypotensive effect of atenolol (ICI 66 082) a new beta adrenergic blocking agent Acta Med Scand 199 257 1976
11 Hansson L Olander R Åberg H Malmcrona R & Westerlund A Treatment of hypertension with propranolol and hydralazine Acta Med Scand 190 531 1971
12 Hansson L Westerlund A Åberg H & Karlberg B E A comparison of the antihypertensive effect of atenolol (ICI 66 082) and propranolol Eur J Clin Pharmacol 9 361 1976
13 Henningsen N C Arborelius M Bulow K Hersvall C Mattiasson I & Ohlsson O Long term treatment of asthmatic and hypertensive patients with atenolol (Tenormin®) (abstract) Swedish Medical Association Assemblee Stockholm 1977
14 Henningsen N C Hersvall C Jameson S Rosenhall L & Åberg H Atenolol (ICI 66 082)—a new selective beta 1 adrenergic blocking agent in the treatment of hypertension in patients with obstructive lung disease Opusc Med 60 1976
15 Karlberg B E Kågedal B Tegler L & Tolagen K Renin concentrations and effects of propranolol and spironolactone in patients with hypertension Br Med J IV 251 1976
16 Katila M & Frick M H Combined dihydralazines

Table III *Presentation of 18 patients who discontinued treatment because of possible side effects*

Type of side-effect	No and sex of pats		No of pats who improved after cessation of treatment
Bradycardia	1 ♂		1
Cold extremities	1 ♂		1
Muscle fatigue	3 ♂		3
Gastrointestinal troubles	1 ♂	1 ♀	2
Shortness of breath fatigue		1 ♀	1
Psychological disorders (fatigue depression headache)	3 ♂	3 ♀	0
Skin reactions (1 eczema seborrhoicum 1 eczema nummulare not provocated)	1 ♂	1 ♀	2
Pathological liver tests	1 ♂		0
Pregnancy		1	–
	18		10

subgroup atenolol+spironolactone (Aldactone®) we consistently saw very good effects on both systolic and diastolic pressure The same experience has been reported in other studies where β blockers have been used in combination with spironolactone (25) Patients who received atenolol and thiazides also showed quite good results but in this subgroup the average BP drop before treatment with atenolol was relatively small and the good result was mainly due to atenolol The combination therapy consisting of β blocker and vasodilator—mainly hydralazine—has a well established effect (11 16)

Patients with moderate but long standing or severe hypertension need in general at least two and very often three or four drugs to become normotensive *Only 8 (9%) of all these atenolol treated* patients did not reach the target diastolic BP (≤100 mmHg) but as they all fell from very high pressure levels (ΔBP>10%) we nevertheless consider them to be responders

Side effects on previous β-blocking therapy

In 51 patients treated previously with another β blocker—alone or in combination with other medication—we changed to atenolol As can be seen in Table II the main reason was suspected or clear side effects from the CNS (fatigue depression nightmares hallucinations or insomnia) in 33 cases Almost all the patients with nightmares *hallucinations and insomnia responded* well to the change but in the patients whose only symptoms were fatigue or depression the result was not so clear about 50% responding well In many of the *patients with nightmares hallucinations insomnia* and/or fatigue the BP response to the previous non selective β-blocker therapy had not been sufficient and we saw a remarkably good pressure response in them In all 6 patients who experienced bronchospastic symptoms we saw a relief of these symptoms (Table II)

Among all these 104 atenolol treated patients only three shifted because of cold extremities or Raynaud s phenomenon They all improved but it should be added that in most patients who complained constantly of such types of side effects we stopped β blocker treatment and shifted to other types of drugs i e spironolactone diuretics or *vasodilators One female patient (aged 45) gained* quite substantially in weight (10 kg) during treatment with propranolol but this trend was clearly broken and her weight turned back to the initial after the change to atenolol

Atenolol induced side effects and drop outs

In 17 (9 6%) patients we discontinued treatment with atenolol because of suspected or clear side effects (Table III) In the patients who complained of psychological disorders (fatigue depression) these seem to have been a symptom rather than a side-effect as we did not see any improvement after the cessation of atenolol Two of these patients also complained of similar side effects during therapy with propranolol and alprenolol

Two patients developed dermal reactions which probably were induced by atenolol but in neither case was the reaction like those seen during therapy *with practolol One of the patients showed an acute* seborrhoic eczematic reaction in the skull one week after initiation of atenolol treatment and was clearly better after withdrawal and local treatment After one month we provoked with atenolol and the *same thing happened One patient who previously* had shown some dermal reaction to hydralazine showed an acute nummular eczematic reaction all over the body After cessation of atenolol and institution of local treatment the reaction almost disappeared In this case we made extensive investigations with skin biopsy (routine staining and immunofluorescent technique) ANF LE cell test electrophoresis and repeated routine metabolic parameters but no signs of an autoimmunological

disease could be found and the biopsies showed only a non specific toxic dermatitis

In a total of 10 patients we registered disappearance of symptoms after cessation of atenolol in five of them the side effects were quite non specific and more related to the β blockade i e bradycardia cold extremities and muscle fatigue

DISCUSSION

Atenolol has in several studies shown its usefulness in the treatment of hypertension and this is in line with experience from other β blockers Atenolol seems to lack side effects from the CNS when given in the normal dosage range and therefore seems to have an advantage over the more lipid soluble and/or non selective β blockers Zacharias et al (26) have described many side effects during therapy with propranolol and about 10% of their patients complained of suspected CNS side effects but treatment was discontinued in only a few cases We have found that about 20–25% of all propranolol treated patients sooner or later develop some CNS related side-effects and quite many of them begin to complain of these after several months of treatment—in five cases in this material after at least one year s treatment with propranolol We also believe that the patient s compliance for attenuation of therapy and control of BP is very much based on the lack of side effects during the therapy Very few studies have included a comparison between various β blockers in respect to their CNS side-effects (7)

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REFERENCES

1 Åstrom H & Vallin H Effect of a new beta adrenergic blocking agent ICI 66 082 on exercise hemodynamics and airway resistance in angina pectoris Br Heart J 36 1194 1974
2 Borgesen S E Nielsen J L & Moller C E Prophylactic treatment of migraine with propranolol Acta Neurol Scand 50 651 1974
3 Case D E Personal communication
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5 Felix R H Ive F A & Dahl M G C Cutaneous and ocular reactions to practolol Br Med J IV 321 1974
6 Formgren H Practolol in the treatment of tachyarrhythmias in patients with bronchial asthma Am Heart J 84 710 1972
7 Hansson L Beta adrenergic blockade in essential hypertension Effects of propranolol on hemodynamic parameters and plasma renin activity Acta Med Scand (Suppl) 550 1 1973
8 Hansson L Henningsen N C Karlberg B E Åberg H Jameson S Malmcrona R & Hersvall C Hypotensive action of ICI 66 082 a new beta adrenergic blocking agent Int J Clin Pharmacol 10 206 1974
9 Hansson L Karlberg B E Åberg H Westerlund A Henningsen N C & Jameson S Clinical evaluation of atenolol in hypertension Clin Sci Mol Med 51 513 1976
10 Hansson L Karlberg B E Åberg H Westerlund A Jameson S & Henningsen N C Long term hypotensive effect of atenolol (ICI 66 082) a new beta adrenergic blocking agent Acta Med Scand 199 257 1976
11 Hansson L Olander R Åberg H Malmcrona R & Westerlund A Treatment of hypertension with propranolol and hydralazine Acta Med Scand 190 531 1971
12 Hansson L Westerlund A Åberg H & Karlberg B E A comparison of the antihypertensive effect of atenolol (ICI 66 082) and propranolol Eur J Clin Pharmacol 9 361 1976
13 Henningsen N C Arborelius M Bulow K Hersvall C Mattiasson I & Ohlsson O Long term treatment of asthmatic and hypertensive patients with atenolol (Tenormin®) (abstract) Swedish Medical Association Assemblee Stockholm 1977
14 Henningsen N C Hersvall C Jameson S Rosenhall L & Åberg H Atenolol (ICI 66 082)—a new selective beta 1 adrenergic blocking agent in the treatment of hypertension in patients with obstructive lung disease Opusc Med 60 1976
15 Karlberg B E Kågedal B Tegler L & Tolagen K Renin concentrations and effects of propranolol and spironolactone in patients with hypertension Br Med J IV 251 1976
16 Katila M & Frick M H Combined dihydral

and propranolol in the treatment of hypertension Int J Clin Pharmacol 4 111, 1970
7 MacDonald A G & McNeill R S A comparison of the effect on airway resistance of a new beta blocking drug ICI 50 172 and propranolol Br J Anaesth 40 508 1968
18 Migraine—Mechanism and Management Symposium Brisbane Sydney and Melbourne June 1976 Abstracts pp 17–22
9 Morgan T O Anavekar S N Sabto J Louis W J & Doyle A E A comparison of beta adrenergic blocking drugs in the treatment of hypertension Postgrad Med J 50 253 1974
0 Powles, R Shinebourne E & Hamer J Selective cardiac sympathetic blockade as an adjunct to bronchodilator therapy Thorax 24 616 1969
21 Prichard B N C Boakes A J & Day G Practolol in the treatment of hypertension Postgrad Med J (Suppl) 47 84 1971
22 Prichard B N C & Gillam P M S The use of propranolol in the treatment of hypertension Br Med J 2 725 1964
23 — Treatment of hypertension with propranolol Br Med J 1 7 1969
24 Schroder G & Werko L Nethalide a beta adrenergic blocking agent Clin Pharmacol Ther 5 159 1964
25 Wright P Ocular reactions to beta blocking drugs Br Med J IV 577 1975
26 Zacharias F J Cowen K J Prestt J Vickers J & Wall B G Propranolol in hypertension A study of long term therapy 1964–1970 Am Heart J 83 755 1972

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Non-Invasive Assessment of Cardiac Function in Meningitis

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ABSTRACT Non invasive registration of systolic time intervals (STI) and ECG were used in evaluating cardiac function in 12 patients with bacterial and 16 with viral meningitis On admission 14 (50%) of the patients had abnormal STI The preejection period (PEP) was prolonged in 11 patients, while left ventricular ejection time (LVET) was prolonged in two and shortened in four The PEP/LVET index was increased in eight patients At discharge PEP was still prolonged in eight patients seven of these were from the viral meningitis group LVET increased significantly ($p<0.02$) from the first to the last registration In the patients with abnormal PEP and PEP/LVET on admission there was a significant fall ($p<0.05$ and $p=0.02$ respectively) to discharge There was no difference in STI between the patients with viral or bacterial meningitis Eighteen (64%) of the patients had abnormal STI on one or more registration ST–T changes in ECG and prolongation of the Q–T interval were found in three patients Cardiac affection in meningitis seems to occur more often than can be shown by ECG

Cardiac affection may occur in both bacterial and viral infections Pericarditis and myocarditis have been described in patients with meningococcal infections (1 4 13 14) In viral infections especially in mumps meningitis and myocarditis are well known complications (3 15) Most of the studies referred to have been based on conventional ECG interpretations Recently the effects of acute infectious diseases on circulatory function have been examined by resting and exercise ECG (8)

Measurements of systolic time intervals (STI) by ECG phonocardiogram and carotid pulse wave is a simple non invasive method to evaluate left ventricular function Left ventricular ejection time (LVET) and preejection period (PEP) have been shown to correlate well with cardiac output and stroke volume (17) In 68 patients with a wide variety of cardiac diseases PEP/LVET correlated highly ($r=-0.90$) to the angiographically determined left ventricular ejection fraction (9)

In the present study STI and ECG were used to examine left ventricular function in patients with meningitis

PATIENTS

The patient group consisted of 17 men and 11 women aged 10–73 years (mean 27 8)

The diagnosis of bacterial meningitis was based on clinical findings and cerebrospinal fluid changes high cell count with a majority of polymorphnuclear cells low glucose concentration demonstration of bacteria in direct Gram stained smear and bacterial growth on culture media

Bacterial meningitis was diagnosed in 12 patients (Table I) Three patients (nos 8 9 and 11) developed septic shock and one of these (no 8) died Patient 12 was comatose on admission and died later from respiratory and cardiac arrest The patients with bacterial meningitis were treated with antibacterial drugs (penicillin sulphonamides and chloramphenicol) Electrolyte and fluid imbalances were corrected as soon as possible after admission

Viral meningitis was diagnosed in 16 patients (Table II) by clinical findings low cell count with a majority of mononuclear cells absence of bacterial growth on culture media and normal glucose levels in the cerebrospinal fluid Virus identification was made by serological tests The patients with viral meningitis were given analgetics and antipyretics

The mean duration of the hospital stay was 17 days in the bacterial and 11 8 days in the viral meningitis group

METHODS

The systolic intervals were measured from simultaneous registrations of carotid pulse wave phonocardiogram and

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Abbreviations ECG=electrocardiogram STI=systolic time interval LVET left ventricular ejection time PEP preejection period QS2=total electromechanical systole

Table I *Individual data and STI in 12 patients with bacterial meningitis*
a=1st registration on admission b=2nd registration on 3rd or 4th day c=3rd registration at discharge N=normal

Pat no	Sex	Age (y)	Infection	Registration	BP (mmHg)	Temp (C)	ECG ST-T segm	ECG Q-T int (sec)	HR (beats/min)	QS2 (%)
1	♀	73	L monocytogenes	a	195/90	39 3	N	0 39	95	96
				b			N	0 39	77	94
				c			N	0 39	79	98
2	♂	71	Unknown	a	110/80	38 3	N	0 39	50	102
				b			N	0 38	51	102
				c			N	0 40	55	100
3	♀	15	N meningitidis	a	90/60	38 6	N	0 38	98	98
				b			N	0 38	84	100
				c			N	0 37	97	106
4	♂	16	N meningitidis	a	120/75	37 7	N	0 33	121	113
				b			Neg T	0 36	92	99
				c			N	0 37	83	99
5	♂	11	N meningitidis	a	110/80	38 5	N	0 39	61	90
				b			N	0 38	65	95
				c			N	0 41	98	101
6	♂	46	Unknown	a	125/80	38 3	N	0 26	57	99
				b			N	0 36	51	100
				c			N	0 36	76	10?
7	♂	46	E coli	a	120/80	37 2	N	0 42	85	103
				b			N	0 38	82	104
				c			N	0 42	75	107
8	♂	59	S aureus	a	110/75	40 4	N	0 38	132	108
9	♀	24	N meningitidis	a	140/80	37 1	N	0 39	132	110
				b			N	0 35	104	105
				c			N	0 37	94	104
10	♂	39	N meningitidis	a	140/75	40 2	N	0 43	94	106
				b			N	0 38	80	94
				c			N	0 41	60	96
	♀	17	N meningitidis	a	75/50	39 8	N		130	108
				b			N		101	98
				c			N	0 40	90	101
12	♂	18	N meningitidis	a	140/70	39 3	N	0 41	140	127

ECG as described by Weissler et al (17) The intervals measured were total electromechanical systole (QS2) from the beginning of the QRS complex in ECG to the second heart sound in phonocardiogram and LVET from the beginning of the upstroke to the dicrotic notch in the carotid pulse wave PEP was calculated as the difference between QS2 and LVET QS2 LVET and PEP were corrected for heart rate by calculating percentages of the normal values at different heart rates The normal values described earlier (5) were based on measurements from 25 women and 25 men PEP/LVET was calculated from the primary measured values

The first registrations of STI were made in all patients less than 24 hours after admission the second on the third or fourth day and the last shortly before discharge In the two patients who died only the first registrations were carried out

ECG was registered simultaneously with STI The Q-T interval was measured from the beginning of the QRS complex to the end of the T wave in ECG The intervals were measured to the nearest 0 01 sec and corrected for heart rate by the formula

$$Q\text{-}T_{corrected} = Q\text{-}T_{measured} / \sqrt{R\text{-}R \text{ interv (sec)}} \quad (6)$$

In one patient (no 11) it was possible to measure this interval only on the last registration

Statistical analyses were performed by linear regression and Wilcoxon s tests for two samples and for paired differences

LVET (%)	PEP (%)	PEP/LVET
85	129	0 5_0
89	110	0 420
98	89	0 300
103	97	0 320
100	108	0 365
98	107	0 375
94	111	0 405
93	121	0 445
104	114	0 375
102	160	0 545
89	131	0 510
98	105	0 370
88	98	0 380
87	120	0 415
96	117	0 415
97	106	0 375
95	116	0 420
103	103	0 345
95	127	0 460
97	125	0 440
106	113	0 380
83	184	0 770
96	158	0 545
105	107	0 355
102	113	0 380
106	108	0 350
93	99	0 365
91	110	0 415
111	99	0 305
104	91	0 300
99	108	0 380
120	146	0 425

RESULTS

On admission 14 patients (50%) had abnormal STI Eight of these had bacterial meningitis (Fig 1) QS2 was prolonged (>108%) in three patients LVET was prolonged (>110%) in two and shortened (<90%) in four patients PEP was prolonged (>115%) in 11 patients and shortened (<85%) in one PEP/LVET was increased (>0 45) in eight patients The two patients who died had extreme pathological values on admission (Table I)

On the second registration STI was abnormal in 11 patients LVET was shortened in three PEP prolonged in 10 and PEP/LVET increased in three patients

On the last registration STI was still pathological in eight patients Seven of these were from the viral meningitis group PEP was prolonged in eight patients and PEP/LVET increased in one

Four patients who had normal values on admission developed pathological values during the hospital stay

LVET showed a significant rise ($p<0\ 02$) from the first to the last registration The patients with pathological values on admission displayed a significant fall from the first to the last registration in PEP ($p<0\ 05$) and PEP/LVET ($p=0\ 02$) From the first to the last registration the mean values for LVET increased while PEP and PEP/LVET decreased (Table III)

There were no significant differences at any registration between the patients with bacterial and viral meningitis with respect to the systolic intervals

ST–T changes in ECG were found in three patients (Tables I and II) The Q–T interval was prolonged (>0 42 sec) in three patients Two of these had a prolongation on admission and one on the last registration One of the patients with ST–T changes had prolongation of the Q–T interval The correlations between Q–T and the systolic intervals were poor (Q–T to QS2 $r=0\ 05$ Q–T to LVET $r=-0\ 17$ Q–T to PEP $r=-0\ 11$ $n=78$)

DISCUSSION

Cardiac affection in infectious diseases can be explained by various mechanisms (7) In bacterial infections toxin may cause myocardial damage or the microorganisms may directly invade the myocardium In patients who died from myocarditis caused by meningococcemia herpex simplex and chickenpox infiltration of leukocytes and degeneration of myofibers were found post mortem (1) The possible side-effects of drugs and the effects of electrolyte imbalance and acidosis must be considered However it has been stated that the circulatory failure in acute infectious diseases is most often caused by peripheral pooling of blood and reduced venous return to the heart (7)

ECG changes have been found in 203 of 2 310 patients with acute infectious diseases (2) but myocarditis has also been shown post mortem when the ECG had been normal (1) ST–T changes in

Table II *Individual data and STI in 16 patients with viral meningitis*

a=1st registration on admission b=2nd registration on 3rd or 4th day c=3rd registration at discharge N=normal

Pat no	Sex	Age (y)	Infection	Registration	BP (mmHg)	Temp (°C)	ECG ST-T segm	ECG Q-T int (sec)	HR (beats/min)	QS2 (%)
13	♂	22	Mumps	a	130/70	40 5	N	0 40	88	95
				b			N	0 38	68	95
				c			N	0 40	71	102
14	♀	10	Influenza A	a	130/70	39 5	N	0 41	111	106
				b			N	0 39	79	102
				c			N	0 41	85	104
15	♀	17	Mumps	a	140/70	38 3	N	0 38	96	102
				b			N	0 40	62	96
				c			N	0 38	54	100
16	♀	40	Unknown	a	150/85	37 7	N	0 39	63	101
				b			N	0 40	59	105
				c			N	0 40	68	106
17	♂	22	Mumps	a	130/60	39 0	N	0 34	60	101
				b			N	0 38	52	103
				c			N	0 38	50	97
18	♂	11	Mumps	a	115/60	37 5	N	0 42	71	100
				b			N	0 39	61	102
				c			N	0 40	77	101
19	♂	20	Mumps	a	135/75	38 2	N	0 36	61	95
				b			N	0 38	70	98
				c			N	0 39	73	100
20	♂	21	Mumps	a	130/75	37 3	Neg T	0 36	76	103
				b			Neg T	0 36	83	100
				c			N	0 25	90	103
[illegible]	♂	34	Coxsackie B	a	130/80	37 9	N	0 37	64	95
				b			N	0 36	61	98
				c			N	0 40	61	94
[illegible]	♂	12	Unknown	a	110/80	37 6	Isoel T	0 44	94	101
				b			N	0 44	72	100
				c			N	0 43	101	109
23	♀	28	Mumps	a	120/80	37 5	N	0 38	73	102
				b			N	0 37	55	107
				c			N	0 35	59	105
24	♀	33	Mumps	a	130/90	39 7	N	0 39	74	99
				b			N	0 39	54	106
				c			N	0 36	61	103
25	♂	10	Mumps	a	120/70	39 4	N	0 33	107	98
				b			N	0 35	91	99
				c			N	0 37	64	95
26	♀	26	Coxsackie B	a	110/70	39 0	N	0 37	81	101
				b			N	0 38	61	104
				c			N	0 39	70	104
27	♀	19	Unknown	a	120/80	39 2	N	0 41	107	98
				b			N	0 40	100	100
				c			N	0 44	84	105
28	♂	17	Unknown	a	160/70	39 9	N	0 41	104	96
				b			N	0 40	69	94
				c			N	0 42	58	97

LVET (%)	PEP (%)	PEP/LVET
93	98	0 365
93	103	0 380
98	118	0 415
96	138	0 500
100	109	0 375
100	118	0 405
93	127	0 470
96	98	0 350
103	89	0 295
99	105	0 365
100	123	0 420
103	115	0 380
99	106	0 370
101	109	0 370
98	104	0 360
95	114	0 410
101	106	0 360
102	97	0 325
91	104	0 390
95	108	0 390
103	94	0 315
96	120	0 430
92	126	0 470
95	126	0 455
92	104	0 385
96	103	0 370
99	78	0 270
95	119	0 430
100	120	0 425
104	125	0 415
98	113	0 395
105	115	0 375
105	108	0 355
96	108	0 390
105	108	0 350
96	126	0 450
94	111	0 405
93	117	0 350
95	96	0 350
83	154	0 635
90	148	0 565
98	123	0 430
94	111	0 405
97	94	0 395
106	104	0 340
103	79	0 265
94	94	0 345
97	96	0 340

ECG were found in three of the present patients and the Q–T interval was prolonged in three In meningococcal infections this interval has been found prolonged in one while it was normal in 112 patients (18) Eighteen (64%) of the patients in this study had abnormal STI on one or more occasion Cardiac affection therefore seems to occur more often in meningitis than can be shown by conventional ECG interpretations

The most common findings in this study were shortened *LVET* prolonged *PEP* and increased PEP/LVET These changes in STI can be explained by a reduction in cardiac contractility which tends to prolong PEP and increase PEP/LVET (12) When reduction in cardiac contractility leads to reduction in stroke volume LVET will be shortened (11) However the findings could be explained equally well by a reduction of venous return (12) Pressure in central vessels was not measured in this study However in both the patients who had systolic BP below 100 mmHg on arrival STI was nearly normal In experimental septic shock peripheral pooling of blood and reduced venous return have been shown (10) Three of the patients in this study developed septic shock and others may have been in a preshock state The abnormal STI may not therefore have been caused solely by reduced left ventricular performance but could be secondary to other mechanisms

There was a tendency to normalization of PEP and PEP/LVET from the first to the last registration These findings indicate improvement in cardiac function at the time of discharge However PEP was lengthened in eight patients on the last registration In these patients left ventricular dysfunction was still present although a reduction of plasma volume during bed recumbency has been

Table III *STI on three registrations (mean ±1 S D)*

Registration	*N*	QS2 (%)	LVET (%)	PEP (%)	PEP/LVET
1st	28	101 9	96 3	119 1	0 429
		7 1	7 8	23 5	0 103
2nd	26	99 9	96 3	112 4	0 403
		3 9	5 2	12 4	0 058
3rd	26	101 8	99 9	107 6	0 371
		3 8	3 9	12 4	0 047

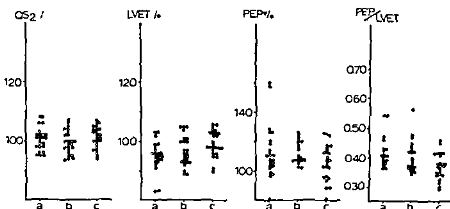


Fig 1 STI in 28 patients with bacterial (O) or viral (●) meningitis *a* = On admission *b* = 3rd or 4th day *c* = at discharge

shown (16) and might partly explain the prolongation in PEP on this registration

On admission eight of the 14 patients with abnormal STI had bacterial meningitis On discharge seven of the eight patients with abnormal STI were from the viral meningitis group The duration of the hospital stay differed between the two groups of patients and this finding indicates cardiac dysfunction on discharge in some of the patients from the viral meningitis group while normalization occurred in a majority of those with bacterial meningitis

This study has shown a reduction of cardiac function in patients with meningitis The high frequency reduced left ventricular performance in these patients may be explained by various mechanisms discussed above The findings indicate careful monitoring of cardiac function in patients with meningitis When infusion of large fluid volumes is necessary STI can be a useful supplement to clinical examination and measurements of pressure in central vessels

REFERENCES

1 Bell R W & Murphy W M Myocarditis in young military personnel Am Heart J 74 309 1967

2 Bengtsson E Electrocardiographic studies in patients with abnormalities in serial examinations with standard leads during acute infectious disease Acta Med Scand 159 395 1957

3 Bengtsson E & Orndahl G Complications of mumps with special reference to the incidence of myocarditis Acta Med Scand 149 381 1954

4 Bonnyns M Delange F Mockel J Balthazar E Dachy A & Cornil A Les complications cardiaques des meningococcies Acta Cardiol 24 131 1969

5 Brubakk O & Overskeid K Systolic time intervals in acute myocardial infarction Acta Med Scand 199 33 1976

6 Chung E K In Electrocardiography p 45 Harper & Row Hagerstown 1974

7 Fine I The cardiovascular system in acute infectious disease Calif Med 79 311 1953

8 Friman G Effects of acute infectious disease on circulatory function Acta Med Scand (Suppl) 592 1976

9 Garrard C L Weissler A M & Dodge H T The relationship of alterations in systolic time intervals to ejection fraction in patients with cardiac disease Circulation 42 455 1970

10 Hinshaw L B Peripheral and cardiac factors in experimental septic shock In The fundamental mechanisms of shock (ed L B Hinshaw & B G Cox) p 363 Plenum Press New York 1972

11 Kesteloot H On the clinical value of mechanocardiography Eur J Cardiol 4 393 1976

12 Lewis R P Leighton R F Forester W R & Weissler A M Systolic time intervals In Noninvasive cardiology (ed A M Weissler) p 301 Grune & Stratton New York 1974

13 Morse J R Oretsky M I & Hudson J A Pericarditis as a complication of meningococcal meningitis Ann Intern Med 74 212 1971

14 Penny J L Grace W J & Kennedy R J Meningococcic pericarditis Am J Cardiol 18 281 1966

15 Roberts W C & Fox S M Mumps of the heart Circulation 32 342 1965

16 Vogt F B & Johnson P C Plasma volume and extracellular fluid volume change associated with 10 days bed recumbency Aerospace Med 38 21 1967

17 Weissler A M Harris W S & Schoenfeldt C D Bedside technics for the evaluation of ventricular function in man Am J Cardiol 23 577 1969

18 Wolf R E Unusual electrocardiogram in meningococcal disease Am Heart J 76 293 1968

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Menopausal Age ın Relatıon to Smokıng

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ABSTRACT A population study of women revealed more smokers among 50 year old postmenopausal women than among women of the same age who still menstruated The difference was statistically significant The postmenopausal smokers had on average smoked as long as or longer than the smokers who still menstruated The higher number of smokers among postmenopausal women could thus not be explained by these women starting to smoke in connection with the menopause Non smoking women were on average heavier than smoking women Previous studies indicate that an increased amount of adipose tissue might delay the menopausal age It is therefore possible that the difference in menopausal age between smoking and non smoking women might be explained either by a delayed menopause in non smoking women due to an increased amount of adipose tissue in these women or by a precocious menopause in smokers due to toxic effects from smoking Probably both factors are of importance, but our results indicate that smoking per se is the main factor The increased number of smokers among women with precocious menopause can probably explain part of the overrepresentation of women with precocious menopause among those who have myocardial infarction

Key words menopausal age smoking body weight population study

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In recent years there have been several reports on the negative effect of smoking and of cigarette smoking in particular There has been evidence that cigarette smoking gives rise to an increased risk of bronchial cancer chronic bronchitis and ischaemic heart disease Unwanted effects on the foetus during pregnancy have also been reported e g reduced growth of the foetus decreased weight at birth and an increased number of malformations (7 15)

In addition to effects on the vessels and direct toxic effects on various organs cigarette smoking may have effects mediated via the ovarian hormonal system Thus decreased secretion of testosterone has been reported in smokers which was quickly normalized when tobacco smoking was withdrawn (6) An earlier menopausal age has been reported in smokers compared to non smokers (5 8 9 13) The reason for a precocious menopause in smoking women may be an early ovarian insufficiency However little is known about the reasons for an early menopause or an early ovarian insufficiency in smoking women

An association has been reported between body weight and menopausal age (9) and between body weight and smoking habits (11 17) Body weight will therefore be taken into consideration in this communication

The association between an early menopause and smoking found by previous investigators was considered by us to be so interesting that we determined to study this question further in a population sample of women in age strata near mean menopausal age The question is of particular interest as there also seems to be an association between precocious menopause and myocardial infarction and between smoking and myocardial infarction as found by other investigators as well as by us (2) The hypothesis may therefore be put forward that an association between early menopause and myocardial infarction could be explained by an increased number of smokers among women with precocious menopause

STUDY POPULATION AND METHODS

A population study of women was carried out in Goteborg Sweden during the years 1968–69 (3) Altogether 1 462 women in the age strata 38 46 50 54 and 60 were studied Women born on certain predetermined dates (multiples of 6 6 12 18 etc) were invited to the study Due to the method of sampling and a high participation rate 90 1% the participants were representative of women in Goteborg in the age strata studied The study was carried out mostly during a 12 month period and those born at the beginning of the year were called first In this

Table I *Age distribution of women included or not included in the study*

Age (y)	Total no of participants	Excluded: No menstruations for 2–5 mo	Excluded: Artificial menopause	Included: Premenopausal	Included: Postmenopausal
46	431	12	31	349	39
50	398	34	35	168	161
54	179[a]	5	18	10	146

[a] One woman who had never had any menstruations is excluded

way the influence of variation in age within each age stratum was reduced to a minimum Further details about sampling participation rate characteristics of participants and non participants have been given previously (3)

The participants passed various examination stations in a fixed order (3) one of which consisted of an examination and interview by an internist In this interview the women were asked about smoking habits Body weight was estimated to the nearest 0 1 kg with the women wearing only briefs Information about the date of the last menstruation was obtained by questionnaire Women who had menstruated during the last two months were defined as premenopausal Those who had not had menstruations during the last five months were defined as postmenopausal We concentrated in this study on the 50-year old women (mean age at the time of the examination 50 55 years S D 0 20) This age stratum consisted of 398 women and information about both smoking habits and menopausal state was obtained from all of them Thirty five of these women had stopped menstruating as a consequence of surgery (bilateral oophorectomy in 15 hysterectomy in 20) These women were excluded as well as 34 others who had not had any menstruations for two months or more but within the last five months The numbers of still menstruating or premenopausal ($n=168$) and spontaneously postmenopausal 50-year-old women ($n=161$) were similar

Information will also be given about the 46-year old women the majority of whom were still menstruating and about the 54 year old women most of whom were postmenopausal Numbers of women included in or excluded from the study are given in Table I

Statistical methods Mean values and S D were calculated according to conventional methods The hypothesis of no differences in frequencies between groups was tested by means of the χ^2 test Significance of differences between mean values was tested with Student s t test or analysis of variances (18) The differences were considered statistically significant for $p<0\ 05$

RESULTS

As shown in Table II there were more smokers among the 50-year old women who were postmenopausal than among those who still menstruated The difference was statistically significant *A similar trend was found in 54 year old* women but among them the number of premenopausal women was too small to allow definite conclusions There seemed to be no overrepresentation of smokers among the postmenopausal 46-year old women

Of 33 bilaterally oophorectomized women 14 (42%) were smokers and of 51 hysterectomized women 16 (32%) Smoking in these two groups (not included in Table II) thus seemed to be somewhat more common than in premenopausal women and less common than in women of similar age with spontaneous menopause Of 50 women who had their latest menstruation 2–5 months before the examination 21 (42%) were smokers

Table III gives further information about the 50-

Table II *Age distribution of smokers among pre and postmenopausal women*

Age (y)	Premenopausal: Total (n)	Premenopausal: Smokers n	Premenopausal: Smokers %	Postmenopausal: Total (n)	Postmenopausal: Smokers n	Postmenopausal: Smokers %	Statistical significance
46	349	151	43	39	18	46	N S
50	168	43	26	161	80	50	$p<0\ 001$
54	*10*	*3*	*30*	*146*	58	40	N S

N S = not significant

Table III *Smoking habits among premenopausal (n=168) and postmenopausal (n=161) 50 year old women*

	Premenopausal		Postmenopausal	
	n	%	n	%
Non smokers	116	69	71	44
Ex smokers	9	5	10	6
Smokers	43	26	80	50
<15 cig /d	35	21	65	40
≥15 cig /d	8	5	15	9

year-old women It is seen that the number of ex smokers was similar among premenopausal and postmenopausal women Heavy smoking was as common among premenopausal as postmenopausal smokers Thus 8 (19%) of 43 premenopausal smokers were heavy smokers (≥ 15 cigarettes/day) compared to 15 (19%) of 80 postmenopausal smokers

Table IV shows the duration of smoking in premenopausal and postmenopausal 50-year-old smokers If anything there may be a slight trend towards an overrepresentation of postmenopausal women among those who started to smoke early but no difference in statistical significance was found irrespective of the definition of early in this context As Table IV indicates the larger number of smokers in the postmenopausal group could therefore not be explained by debut of smoking in connection with the menopause as very few women had started to smoke in recent years

Body weight seemed to be higher in premenopausal than in postmenopausal women and higher in non smokers compared to smokers (Table V) There were differences of statistical significance according to analysis of variance ($p<0.001$) However when all the groups studied

Table IV *Duration of smoking in premenopausal (n=43) and postmenopausal (n=80) 50 year old smokers*

Duration of smoking (y)	Premenopausal n	Premenopausal %	Postmenopausal n	Postmenopausal %
<5	1	2	1	1
5–9	4	9	0	0
10–19	7	16	8	10
20–29	13	30	28	35
30–39	18	42	43	54

Table V *Body weight (kg) in pre and post menopausal smoking and non smoking 50 year old women (ex smokers excluded)*

	Premenopausal			Postmenopusal		
	n	Mean	S D	n	Mean	S D
Smokers	43	66 0	11 2	80	62 3	10 7
Non smokers	116	69 8	11 0	71	66 7	9 6

were compared by means of Scheffe s method (18) a difference of statistical significance was found only between premenopausal non smokers and postmenopausal smokers

Thus there seemed to be some association between body weight and menopausal state as well as between body weight and smoking habits In order to eliminate the influence of body weight the number of smokers among pre and postmenopausal women was studied in various ranges of body weight (Table VI) There was still an over representation of smokers among postmenopausal women when women of similar body weight were compared

DISCUSSION

On the relation between smoking and menopausal age

In agreement with previous reports (5 8 9 13) we noted that there were more smokers among post menopausal 50-year old women than among pre menopausal women of the same age Consequently more smokers than non smokers had reached the menopause at the age of 50 Because of the small numbers of postmenopausal women in the 46-year-old group and of premenopausal women in the 54-year-old group definite conclusions can be drawn only about those aged 50 in the present study but the results from the women aged 46 and 54 do not contradict the results from the group aged 50 It may be of interest to note that smoking was almost as common in premenopausal as in post menopausal 46-year-old women As stated above the group of premenopausal women of this age is small but the results may indicate that smoking has a moderate influence on menopausal age as a difference was detected in the age group 50 years—which was found to be the approximate median menopausal age—but not in the age group 46 rs A similar trend was noted by Jick et al (1

Table VI *Number of smokers among pre- and postmenopausal 50 year old women in various ranges of body weight*

Body weight (kg)	Premenopausal Total (n)	Premenopausal Smokers n	Premenopausal Smokers %	Postmenopausal Total (n)	Postmenopausal Smokers n	Postmenopausal Smokers %	Statistical significance	
<50	4	3	75	9	7	78	–	
50 0–54 9	10	3	30	19	12	63	N S	
55 0–59 9	25	6	24	28	15	54	$p<0.05$	
60 0–64 9	41	11	27	38	23	61	$p<0.01$	$p<0.001$
65 0–69 9	31	7	23	29	11	38	N S	
70 0–74 9	21	6	29	16	4	25	N S	
≥75 0	36	7	19	22	8	36	–	

N S = not significant

Which is the primary factor early menopause or smoking?

The finding of a larger number of postmenopausal women among smokers might be explained in two ways Either smoking women do have an earlier menopause than non smoking or women start smoking as a consequence of the menopause Our results clearly show that the second possibility is not valid Very few women had started smoking in recent years Precocious menopause is thus a consequence of smoking and not vice versa This conclusion is in agreement with the results presented by Jick et al (13)

an early menopause in smokers be related to other factors than smoking per se?

Previous studies indicate an association between an increase in body weight and late onset of the menopause (9) There are also reports that an increased amount of adipose tissue might give rise to an increased oestrogen production peripherally (14 16) In the present study non smokers were on average heavier than smokers This agrees with our previous results (17) and with those from the Framingham study (11)

It has been shown that there are differences in metabolism between smokers and non smokers Thus an increase in body weight coincident with decreases in oxygen consumption heart rate and protein bound iodine level in blood was noted during the week following cessation of smoking (1 10) Enhanced drug metabolism in cigarette smokers has also been reported (12) It is possible that the hepatic metabolism of oestrogens is also enhanced in smokers if so this might lead to an earlier fall of oestrogen levels in smokers than in non smokers The possibility thus exists that the non smokers have aquired an increased amount of adipose tissue and that this excess might prolong the premenopausal age rather than this age being shortened by smoking due to toxic or liver inducing effects It is difficult to say whether a prolonging factor in non smokers or a shortening factor in smokers is the sole or the predominant cause Probably both factors are of importance Our results showing an overrepresentation of smokers among postmenopausal compared to premenopausal women of similar body weight indicate that an earlier menopause in smoking women is at least partly unrelated to the amount of fat tissue in the body

Can the predominance of smokers among women with precocious menopause explain the higher morbidity rate of ischaemic heart disease among them?

As mentioned in the introduction an increased morbidity in ischaemic heart disease among women with precocious menopause and among women who are smokers has been reported in several studies It should be noted that the relationship between early menopause and myocardial infarction is rather weak while that between smoking and myocardial infarction is stronger (2) It therefore seems possible that a higher incidence of myocardial infarction in women with precocious menopause could be explained by a larger number of smokers in this group of women The results from the present study make

this possibility probable but the present material does not allow definite conclusions However when evaluating the combined influence of smoking and menopausal age in a group of women who had myocardial infarction in the same area (2) it was concluded that the larger number of smokers among women with early menopause seemed to explain only part of the higher incidence of myocardial infarction among them (4)

REFERENCES

1 Batterman R C The biologic effects of tobacco (ed E L Wynder) p 140 Little Brown & Co Boston 1955
2 Bengtsson C Ischaemic heart disease in women A study based on a randomized population sample of women and women with myocardial infarction in Goteborg Sweden Acta Med Scand (Suppl) 549 1973
3 Bengtsson C Blohmé G Hallberg L Hallstrom T Isaksson B Korsan Bengtsen K Rybo G Tibblin E Tibblin G & Westerberg H The study of women in Gothenburg 1968–1969—a population study General design purpose and sampling results Acta Med Scand 193 311 1973
4 Bengtsson C & Lindquist O Coronary heart disease—during the menopause In Coronary heart disease in young women (ed M F Oliver) Churchill Livingstone Edinburg 1978
5 Bernhard P Die Wirkung des Rauchens auf Frau und Mutter Munch Med Wochenschr 104 1826 1962
6 Briggs M H Cigarette smoking and infertility in men Med J Aust 1 616 1973
7 Butler N R Goldstein H & Ross E M Cigarette smoking in pregnancy Its influence on birth weight and perinatal mortality Br Med J 2 127 1972
8 Daniell H W Osteoporosis and smoking JAMA 221 509 1972
9 – Osteoporosis of the slender smokers Vertebral compression fractures and loss of metacarpal cortex in relation to postmenopausal cigarette smoking and lack of obesity Arch Intern Med 136 298 1976
10 Glauser S C Glauser E M Reidenberg M M Rusy B F & Tallander R J Dietabolic changes associated with the cessation of cigarette smoking Arch Environ Health 20 377 1970
11 Gordon T Kannel W B Dawber T R & McGee D Changes associated with quitting cigarette smoking The Framingham Study Am Heart J 90 322 1975
12 Hart P Farrell G C Cooksley W G E & Powell L W Enhanced drug metabolism in cigarette smokers Br Med J 2 147 1976
13 Jick H Porter J & Morrison A S Relation between smoking and age of natural menopause Lancet 1 1354 1977
14 McDonald P C van de Wiele R M & Lieberman S Precursors of the urinary 11-desoxy 17 keto steroids of ovarian origin Am J Obstet Gynecol 86 1 1963
15 Mulcahy R & Knaggs J F Effect of age parity and cigarette smoking on outcome of pregnancy Am J Obstet Gynecol 101 844 1968
16 Nimrod A & Ryan R J Aromatization of androgens by human abdominal and breast fat tissue J Clin Endocrinol Metab 40 367 1975
17 Noppa H Bengtsson C Bjorntorp P Smith U & Tibblin E Overweight in women—metabolic aspects The population study of women in Goteborg 1968–1969 Acta Med Scand 203 135 1978
18 Scheffe M The analysis of variance Wiley New York 1959

Table I *Frequency of dosage schedules and routes of administration (n=1653)*

	%
Dosage schedules	
Single dose (nurse)	17
Single dose (physician)	6
As required	12
Once daily	20
Twice daily	16
Three times daily	23
Four times daily (every 6th hour)	2
Five to eight times daily	1
Varying	1
Others	1
?	1
Routes	
Oral	81
Rectal	3
Local	1
Parenteral	14
Others or not stated	1

starting changing and terminating the therapy 2) All drug therapy should be registered in a single original document prescription sheet which also should be used as the nurses working document during the dispensing and administration of drugs 3) The medications should be given with exact and rational timing according to pharmacokinetic iciples 4) All administered doses should be by the nurse and if a patient misses a dose reason should be stated The present paper at s to evaluate this system

MATERIAL AND METHODS

The study took place in two wards (out of six) of the Department of Internal Medicine at Huddinge University Hospital Karolinska Institutet The wards contain 22 beds each Patients with malignant blood disorders (17%) and general medical patients except those needing intensive coronary or respiratory care are referred to these wards

Recording and administration of drugs in the wards

Within the wards all drug therapy was recorded in the test system Apart from the usual items (name of drug date started dosage dosage form frequency route of administration date discontinued and signature of prescriber) the indications for the prescriptions and the reasons for stopping or changing each prescription were stated on the "prescription sheet" No transcription steps were permitted

Using a modification of the Aberdeen system a "drug administration sheet was linked to the prescription sheet" (4) All doses administered (observed dose ingestion) were recorded in code together with the time of administration (±30 min) for each drug and signed by the nurses If a prescribed drug dose was not taken by or injected to the patient the nurses stated the reason in a separate column

Self administration

Some drugs—mostly antacids nitroglycerin dermatological agents eye and ear drops—were given to the patients for self administration In these cases the name of the drug the reason for the therapy and dosage intervals (where applicable) were written on a special form which was given to the patient together with a suitable amount of the drug The patient was instructed to note the amount of the drug ingested on a 24 hour schedule so that the doctors could easily see how the therapy was used

Nurse prescriptions

The National Board of Health and Welfare in Sweden allows nurses to administer single doses of some drugs according to a general prescription (11 15) These drugs chosen by the chief physician of the department are listed together with specific instructions for their usage The list may contain laxatives cough medicines some analgesics sedatives hypnotics and minor tranquillizers In this study multiple doses of the same drug given by the nurses on the same indication to the same patient were regarded as one nurse prescription

Times of administration

After a pilot study in 1974 the routine times of drug administration were reduced from four to three for practical reasons to 08 14 and 20 o clock When the pharmacokinetic profile of the drug or the clinical situation called for other dosage intervals these were stated as every 6th hour or 8th hour etc

Data analysis

When the patients had been discharged all data were edited for completeness by a research nurse coded and referred to a data sheet from the drug prescription sheet

Table II *Error rates of drug prescribing*

	% omitted
Physicians (n=1 367)	
Strength	1
Date started	1
Dose	4
Frequency	1
Route of administration	1
Signature of prescriber	3
Indication for therapy	4
Nurses (n=286)	
Strength	2
Dose	7
Indication for therapy	28

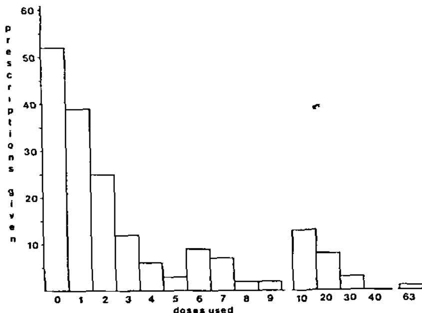


Fig 1 Number of doses used (*n*=850) per as required prescription (*n*=182) Median 1 5 mean 4 7

and from the drug administration sheet Administered doses for each prescription were counted and related to the numbers prescribed Drugs prescribed (*n* 130) on the day of discharge were not included in the estimations of drug therapy in the wards as they mostly were intended for treatment after discharge However 33 of these 130 prescriptions were actually administered on that day I v fluids were not regarded as drugs in this study but drugs added to i v fluids were recorded

Patient data such as age sex weight and some laboratory results were also recorded The data were referred to punch cards and the final editing and calculations were done by a special computer program (written by one of the authors) that also checked the data for completeness plausibility and internal consistency Sample tests revealed an error frequency in preparing the material in the order of 1–2%

Patients

During the study period March 3–June 17 1975 286 patients (331 admissions) came in contact with the system Of these 275 patients/admissions were also discharged during the study period (each admission will be regarded as one patient) The medical record of one of the deceased patients could not be found at the time of data collection All results in this paper are based on the 274 completely covered patients

RESULTS

Prescribing of drugs

The total number of prescriptions was 1653 including nurses prescriptions (17%) Dosage schedules and routes of administration are shown in Table I Prescribing errors related to prescriber are given in Table II For the physicians prescriptions correct drug name starting date and signature were found in 96% and with all parameters included 92% of their prescriptions were correct Strength and dose were stated on 92% of the nurses prescriptions With the therapeutic indications included 64% of their prescriptions were correct

Administration of drugs

Some administration sheets were lost before data collection Most of these were self administration check lists which are not kept routinely These losses are not due to faults in the drug recording system but in patient record handling Complete information about the number of administered doses could be obtained from 96% (1 580/1 653) of all the prescriptions

From the 1 580 prescriptions 17645 doses were recorded as given to the patients Only 5% of these were given by the nurses according to the general prescription For 2% (314) of the doses the nurses signatures (stating dose administration) were missing although they were registered as taken by the patients Four per cent (699) of the doses were not registered as taken by the patients but the reason for this non ingestion was stated for only 391 (56%) For 27 prescriptions 34 doses more than prescribed were given (0 2% of all doses given)

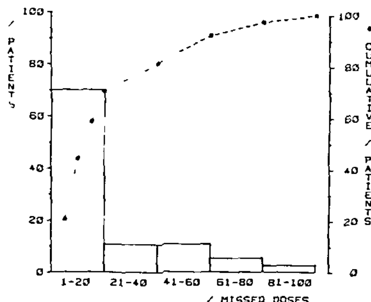


Fig 2 Compliance distribution among 111 patients who missed some doses of their regular prescriptions while in the hospital Among 70% of patients who missed up to 20% of their doses 20% missed not more than 5% 44% not more than 10% and 59% not more than 15% of their doses

Prescribing vs administration of drugs

Of the 1580 prescriptions 7% were never administered in the wards Half of these were prescribed to be given as required Altered clinical status and drug not available in ward were other reasons for not administering a prescription In less than 10% the reason was not obvious The use of as required therapy is shown in Fig 1 About one third of these drugs were never used

Missed doses were found for 41% (111/274) of all the patients corresponding to 27% (280/1047) of the administered regular prescriptions In these cases 9% of the prescribed doses were not recorded as taken by the patients The distribution of missed doses for these prescriptions is shown in Fig 2 Table III shows some characteristics of the patients and Table IV the reasons why the patients did not take their medicines as requested The patients obtained on average 6 0 prescriptions each 4 9 by physicians and 1 0 by nurses If each drug is counted only once per patient irrespective of changes in dose route of administration etc 5 1 drug exposures per patient were prescribed and 4 7 drug exposures per patient were administered

Table III *Characteristics of 226 patients who did or did not miss some doses of regular prescriptions (excludes once only and as required)*

	Missed no doses (n=115)			Missed some doses (n=111)	
	Mean	S D	p<	Mean	S D
Age (y)	57 6	19 6	0 002	65 3	17 5
Days in ward	8 6	6 0	0 001	18 7	15 4
No of prescriptions					
Regular	3 1	1 8	0 001	6 2	3 9
Total	4 7	5 6	0 001	9 7	5 2

Differences between means were tested as described by Armitage (1)

Table IV *Stated reasons for 391 doses not ingested*

	%
Related to routines	
Drug not available in the ward	13
Patient fasting before investigation	9
Patient absent from the ward	3
Related to patient	
Patient not at bedside	8
Patient does not want/refuses	28*
Patient has taken own drug	3
Related to disease	
Nausea/vomiting	6
Difficulties in swallowing	7
Others (coma adverse drug reaction no need etc)	17
Others	5

* 11% clinically justified

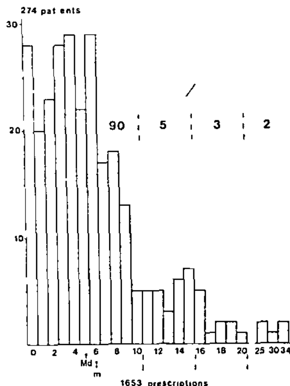


Fig 3 Distribution of 1653 prescriptions in 274 patients Median 5 5 mean 6 0

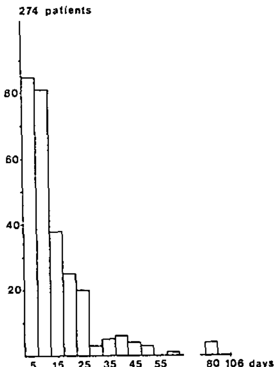


Fig 4 Duration of treatment of 274 patients Median 8 mean 12 Forty three per cent of the patients stayed in the wards for up to one week 72% up to two and 87% up to three weeks

Analysis of drug recording and administration sheets

The drug prescription sheet had space for a total of 20 prescriptions Prescriptions by physicians were started from the top and by nurses from the bottom of the sheet The drug administration sheet covered seven days During the study period (106 days) 90% of the patients received ten prescriptions or less (Fig 3) The mean time spent in the wards was 12 days and about 90% stayed for three weeks or less (Fig 4)

DISCUSSION

Investigations of hospital drug handling were started in Sweden ten years ago (3) A great variety of routines with potential dangers were found This led to the development of new regulations and recommendations from the National Board of Health and Welfare (15) The drug recording system introduced at the start of our hospital in 1972 was still not satisfactory (2) It contained a transcription step which made verbal prescribing without registration possible It lacked a clear instruction manual and no supervision existed This resulted in different applications in different wards In order to improve registration and administration of drugs and to make drug surveillance studies possible a new drug recording system and new drug handling routines were developed and tested in two medical wards

Accuracy of prescribing and recording of drug administration

Using the criteria of correct name of drug correct transcription (1974) starting date and physician s signature the frequency of correct drug prescriptions was 58% in 1974 and 96% with the test system in 1975 The error rates of the physicians were low for each step in the prescription procedure (Tables I–II) Tesh et al (16) found the same order of corresponding errors in a British system without transcriptions

Dose and therapeutic indication were more frequently omitted from the nurse prescriptions than from those of the physicians However an analysis of the actual prescriptions showed that

17/23 omitted strengths or doses concerned fluid expectorants antacids or laxatives Furthermore the indications could in each case be ascertained by checking the type of drug and indications for earlier prescriptions

A system without transcriptions where each ingested dose is recorded has been used since 1965 in hospitals in north-east Scotland However a continuous follow up with feed back (discrepancy lists for each ward) had to be built into the system to keep errors of recording drug administration at an acceptable 2–4% (7) When our test system was introduced seminars were held with the staff and they were given a self instructing manual for the system A research nurse was available as instructor and supervised the maintenance of the system Errors in the recording of drug administration corresponding to the Scottish discrepancies were 2% in our study Thus we consider that abolition of transcriptions increased motivation of the staff and supervision are necessary to improve the accuracy of drug recording systems

Clinical implications of recording drug administration

Non-compliance with prescribed therapy is a well known phenomenon outside hospitals (12 13) Although reports concerning non ingestion of prescribed therapy in hospitals exist (19) the magnitude of the problem is not well established as drug administration is not recorded in most hospitals

Control of drug intake was initially regarded with scepticism by some of the nurses and patients However the nurses attitudes changed as they became aware of several important problems e g 1/4 of the patients needed help with the drug intake and some could not swallow tablets or capsules Their conclusions after the test period were (from a questionnaire) that the test system was safer the extra time well spent and the stated indications for the prescriptions valuable (2) The patients also accepted the increased supervision of drug administration and found that the nurses spent more time informing them about drugs

Irving et al (9) found that 5–32% of psychiatric in patients failed to some degree to take their medication (judged from urine tests) and the frequency correlated to the degree of general supervision of the patients In our study half of the patients who had prescriptions for regular administration (111/226) missed some doses These patients were on average older had more prescriptions and stayed longer in the wards (Table III) On average they missed 9% 46 patients (41%) missed more than 15% of their doses (Fig 2) Unless one knows the frequency of and the reasons for the missed doses evaluation of drug therapy must be impossible

When analysing the reasons for doses not taken (Table IV) some remaining fallacies in the nursing routines were found which have been rectified by now

On average our patients were prescribed 5 1 drug exposures each (and given 4 7) far below figures reported from the US 9 4 and close to those from Scottish hospitals 4 5 (10)

Time vs safety in drug handling

The National Board of Health and Welfare in Sweden instructs the nurses 1) to inform the patients about drugs administered briefly how they act and how they shall be used 2) to get information about positive and negative effects of drugs given 3) to assist the patients with drug intake when needed (15) These demands were not met with the initial routines Before introducing the test system we monitored the drug handling in our wards repeatedly (1974) When administering drugs the nurses placed them on the patient s night table The patient was often absent and drugs from the previous administration round were often found The nurses seldom observed the drug intake One nurse managed to deliver drugs to ten patients in 3 min (2)

With supervision and recording of drug intake the regular drug rounds took on average about 2 min per patient compared to about 1 min without this control We consider this increase to be necessary for improved accuracy and safety in the recording and handling of drugs Moreover the addition of the therapeutic indication to the prescription was appreciated by doctors on call and consultants led to increased pharmacotherapeutic concern and served as a valuable tool in teaching the students (interviews with physicians)

Drug information and drug education

Many studies have shown that the patient s knowledge about drug treatment is inadequate (6 8 12 13) One of the most favourable situations in which to inform patients about their drugs must be

in hospitals The entire health care team must be involved in giving drug information to the patient and checking that it is understood Furthermore the attitudes towards drug handling by the hospital staff will be transferred to the patient and may influence their medication behaviour at home (9)

The self administration list turned out to be a valuable tool in educating the patients about the proper way of administering their drugs By checking these lists we could correct misunderstandings among both nurses and patients e g patients taking antacids with instead of between meals

CONCLUSIONS

Errors are inevitable in all human behaviour To diminish these risks in hospital drug handling an accurate and safe system of drug recording with built in control mechanisms is mandatory The inclusion of the therapeutic rationale for a prescription in the recording system has theoretical and practical advantages that make up for the extra time spent in the prescribing procedure Moreover recording actual drug intake is a prerequisite for the evaluation of drug effects and drug plasma concentrations in clinical trials or practice Finally a thorough system of drug recording can be used in the education and counseling of patients and students Such a system might favourably influence the prescribing habits of students and the medication habits of the patients

ACKNOWLEDGEMENT

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REFERENCES

1 Armitage P Statistical methods in medical research p 122 Blackwell Scientific Publications Oxford London Edinburgh and Melbourne (1971) 1973
2 Bergman U Jacobsen K & Wiholm B E Lakemedelshanteringen i sluten vård—erfarenheter från en jamförelse mellan två system för registrering av lakemedel Tidskrift for Svenges sjukskoterskor (TfSs) 43 no 17 46 1976
3 Bottiger L E Sakrare lakemedelshantering nodvandig på sjukhusen Lakartidningen 66 3585 1969
4 Crooks J Calder G & Weir R D Drugs in hospital J R Coll Physicians Lond 1 233 1967
5 Crooks J Weir R D Coull D C McNab J W Calder G Barnett J W & Caie H B Evaluation of a method of prescribing drugs in hospital and a new method of recording their administration Lancet 1 668 1967
6 Hellstrom K & Leijd B Vad vet patienter om sina sjukdomar och deras medikamentella behandling? Lakartidningen 73 968 1976
7 Henney C R Drug administration I Nurs Mirror 1 52 1976
8 Himmelman C E Lindgren J Mårtensson C Navelér J & Wohrm A Tynneredsprojektet – synpunkter på långtidsmedicinering i oppen vård Lakartidningen 71 4609 1974
9 Irwin D S Weitzell W D & Morgan P W Phenothiazine intake and staff attitudes Am J Psychiatry 127 1631 1971
10 Lawson D H & Jick H Drug prescribing in hospitals An international comparison Am J Publ Health 66 644 1976
11 Medicinalvasendets författningssamling MF 1974 83
12 Parkin D M Henney C R Quirk J & Crooks J Deviation from prescribed drug treatment after discharge from hospital Br Med J 2 686 1976
13 Sackett D L & Haynes R B (ed) Compliance with therapeutic regimens Johns Hopkins University Press Baltimore and London 1976
14 Sjoqvist F Clinical use of drug plasma level determinations In Year book of drug therapy (ed D L Azarnoff L Hollister & D Shand) pp 13–20 Year Book Medical Publishers Chicago and London 1977
15 Socialstyrelsens råd och anvisningar 17 1970 revised 39 1974
16 Tesh D E Beeley L Clewett A J & Walker G F Errors of drug prescribing Br J Clin Pharmacol 2 403 1975
17 Wallace W F M Drugs in hospital Lancet 1 555 1965
18 Watt J A Dorricott N Stewart R A Y & Dawe D P Methods of recording prescriptions in hospital and their effects on transcribed drug lists Br J Psychiatry 122 65 1973
19 Wilson J T & Wilkinson G R Delivery of anticonvulsant drug therapy in epileptic patients by plasma level analyses Neurology 24 7 614 1974

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The Effect of Cimetidine, a New Histamine H_2-Receptor Antagonist, on Renal Function

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ABSTRACT Serum creatinine endogenous creatinine clearance, (^{51}Cr)EDTA plasma clearance and the concentration of β_2-microglobulin in serum and urine were determined in 19 patients before and during treatment with cimetidine for peptic ulcer disease The mean concentrations of creatinine and β_2-microglobulin in serum increased significantly within normal limits after 2 and 6 weeks treatment However, creatinine clearance and (^{51}Cr)EDTA plasma clearance were unchanged during the treatment Thus, the observed increases in serum creatinine and β_2 microglobulin could not be explained by a diminished glomerular filtration rate Inhibited tubular secretion of creatinine may explain the observed increase in serum creatinine during cimetidine treatment Another hypothetical possibility is that a small increase in tubular reabsorption of both creatinine and β_2 microglobulin would account for the observed increases in creatinine and β_2-microglobulin in serum It is concluded that, although statistically significant, the increases in serum creatinine and β_2-microglobulin are small and therefore of little relevance for the patient s treatment with cimetidine

Key words cimetidine histamine H_2 receptor antagonist renal function

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The therapeutic value of cimetidine a histamine H_2 receptor antagonist in peptic ulcer disease has been extensively documented in double blind clinical trials (2 3 9) Its use has increased and recently a promising prophylactic effect has been reported against upper gastrointestinal haemorrhage in transplant recipients with varying degrees of renal insufficiency (11) However statistically significant increases in serum creatinine usually within the normal range are known to occur during the first weeks of treatment with cimetidine (2 3 10) These increases reverse to pretreatment levels during continued administration of cimetidine (3) Burland et al (7) studied the effect of cimetidine on renal function in six healthy volunteers and noted a significant decrease in creatinine clearance after a single dose of 800 mg but no change after 6 days of 1 6 g/d in divided doses They suggested that the increase in serum creatinine is a result of competition by cimetidine for tubular secretion of creatinine

The aim of this study was to further elucidate the effect of cimetidine on renal function in patients with peptic ulcer disease treated with cimetidine for 6 weeks

PATIENTS AND METHODS

Nineteen outpatients 5 women and 14 men mean age 45 years (range 26–63) with endoscopic evidence of peptic ulcer were treated with cimetidine 1 0 g/day for six weeks Renal function was assessed from determinations of serum creatinine serum urea endogenous creatinine clearance (^{51}Cr)EDTA plasma clearance and β_2 microglobulin in serum and urine before and after 2 and 6 weeks of treatment with cimetidine Endogenous creatinine clearance was measured over one or two 24-hour periods The blood sample for serum creatinine was drawn at the end of every 24-hour period Total (^{51}Cr)EDTA plasma clearance was determined with a simplified single injection technique (4) Blood samples were drawn at 180 200 220 and 240 min after i v injection of 100 μCi (^{51}Cr)EDTA (6) The values for creatinine clearance and (^{51}Cr)EDTA plasma clearance were corrected to 1 73 m^2 BSA

β_2 microglobulin in serum and urine was determined by a radioimmunosorbent technique using the kit Phadebas β_2 microglobulin test (Pharmacia Diagnostics Uppsala) Serum creatine phosphokinase was determined and routine urine and blood chemistry was performed during the trial Cimetidine was supplied by Smith Kline and French Laboratories

The statistical analysis was performed with Student s paired t test and the results are given as mean $\pm$ S D

Table I *Renal function and β₂-microglobulin in serum and urine before and during treatment with cimetidine*

Mean ± S D range in parentheses

Time in relation to treatment	Serum creatinine (μmol/l) (n=19)	Urinary creatinine (mmol/24 h) (n=18)	Creatinine clearance (ml/min/1 73 m²) (n=18)	(^{51}Cr)EDTA clearance (ml/min/1 73 m²) (n=17)
Before	83 8±15 0 (60–120)	11 6±3 9 (4 6–16 4)	93 3±24 2 (47–132)	92 3±16 0 (72–121)
2 weeks	90 3±19 4* (63–140)	11 7±3 3 (4 2–16 0)	89 2±17 2 (49–127)	93 8±14 4 (69–122)
6 weeks	89 6±16 2 (70–125)	12 2±4 4 (4 8–19 4)	87 0±26 6 (50–119)	94 9±16 7 (71–127)

p<0 05 **p<0 01 compared to results before treatment

RESULTS

The results of the renal function tests during cimetidine treatment are summarized in Table I All values for serum creatinine before treatment ex cept for one patient were within the accepted ref erence intervals 53–115 μmol/l During cimetidine treatment there was a small increase in the serum creatinine concentrations 8% at two weeks and 7% at six weeks (p<0 05) One patient had an initial serum creatinine concentration of 120 μmol/l and after 2 and 6 weeks of treatment 140 and 125 μmol/l respectively Serum creatinine in all the her patients varied within the reference interval nary creatinine excretion was unchanged during e trial

For one patient the values for creatinine clear ances were omitted in calculations of means and S D due to inadequate urine collections before and during the trial The mean ± S D for the other 18 patients was 93 3±19 7 ml/min/1 73 m² before treatment and there were no significant changes during treatment

For two patients the result of (^{51}Cr)EDTA plasma clearance was invalid due to accidental extravascu lar injection of the (^{51}Cr)EDTA These results were not included in the calculations During cimetidine treatment there were no significant changes in the mean (^{51}Cr)EDTA clearance (^{51}Cr)EDTA clear ance for the two patients with inadequate examina tion before treatment were 103 and 127 ml/min/1 73 m in one and 80 and 79 in the other after 2 and 6 weeks of treatment

A small but significant increase in β microglobulin occurred after 2 and 6 weeks of treat ment within the accepted reference intervals (1 1–2 4 mg/l) However the urinary excretion of β_2 microglobulin did not change

There were no significant changes in serum urea during the trial and proteinuria was not seen The microscopic examination of urine did not yield any relevant findings Mean serum creatinine phosphokinase was unchanged during the trial After two weeks of treatment one patient had a slight increase in serum aspartate aminotransferase and alanine aminotransferase These enzymes had normalized at the end of the trial

DISCUSSION

Previous reports on small but significant increas es in serum creatinine during treatment with cimetidine (2 3 10) were confirmed in this study It was expected that an anticipated small decrease in the glomerular filtration rate would be difficult to demonstrate with the determination of creatinine clearance since the reproducibility of this method is low due to difficulties in obtaining quantitative urine collections for 24 hours (5) In accordance with the unchanged creatinine clearance however the values for (^{51}Cr)EDTA clearance which is a highly reproducible method (5) did not diminish during the trial which strongly supports the opinion that the glomerular filtration rate is not influenced by cimetidine therapy

Creatinine in serum is mainly cleared by glomeru lar filtration but small quantities are also secreted and possibly also reabsorbed in the tubules (1 13) An inhibited tubular secretion of creatinine during cimetidine therapy can therefore not be ruled out

In agreement with Burland et al (7) we did not

Serum β_2 microglobulin (mg/l) ($n=19$)	Urinary β_2 microglobulin (μg/24 h) ($n=18$)
1 9±0 49 (1 4–3 7)	172±178 (23–692)
2 0+0 39* (1 5–3 1)	161±186 (21–790)
2 1+0 51** (1 1–3 5)	290±340 (26–1 440)

find any increase in serum creatine phosphokinase during the trial This indicates that the increase in serum creatinine during cimetidine treatment should not be attributed to any excessive endogenous production of creatinine caused by muscle breakdown which is also supported by the unchanged creatinine excretion in urine

Neither cimetidine nor its sulphoxide metabolite have been shown to have any effect on the determinations of serum and urinary creatinine concentrations (7) which indicates that the elevation of serum creatinine in patients receiving cimetidine is not due to an interference with the analytical procedures

β_2 microglobulin is eliminated from blood via glomerular filtration More than 99 9% of filtered protein appears to be reabsorbed in the renal tubules as only small amounts are excreted in urine (8 14) In renal insufficiency there is a striking correlation between the increase in β_2 microglobulin and creatinine in serum (12) and between the increase in serum β_2 microglobulin and the reduction of inulin clearance (15) In the presence of an unchanged glomerular filtration rate estimated with (^{51}Cr)EDTA clearance it is difficult to give a reasonable explanation for the slight rise in serum β_2 microglobulin Another possible hypothesis is however that a small increase in tubular reabsorption of both creatinine and β_2 microglobulin might explain the observed increases in creatinine and β_2 microglobulin in serum

The results of this study suggest that cimetidine does not cause any clinically significant impairment of the glomerular filtration rate The observed rise within normal limits in mean serum creatinine during cimetidine treatment might be caused by inhibited tubular secretion or increased reabsorption of creatinine Such tubular effects of cimetidine would probably be of minor clinical importance

ACKNOWLEDGEMENT

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REFERENCES

1 Berglund F Urinary excretion patterns for substances with simultaneous secretion and reabsorption by active transport Acta Physiol Scand 52 276 1961
2 Blackwood W S Maudgal D P Pickard R G Lawrence D & Northfield T C Cimetidine in doudenal ulcer Lancet 2 174 1976
3 Bodemar G & Walan A Maintenance treatment of recurrent peptic ulcer by cimetidine Lancet 1 403 1978
4 Brøchner Mortensen J A simple method for the determination of glomerular filtration rate Scand J Clin Lab Invest 30 271 1972
5 Brøchner Mortensen J & Rodbro P Selection of routine method for determination of glomerular filtration rate in adult patients Scand J Clin Lab Invest 36 35 1967
6 — Optimum time of blood sampling for determination of glomerular filtration rate by single injection (^{51}Cr)EDTA plasma clearance Scand J Clin Lab Invest 36 795 1976
7 Burland W L Gleadle R I Mills J G Sharpe P C & Wells A L The effect of cimetidine on renal function In Cimetidine (ed W L Burland and M A Simkins) p 67 Excerpta Medica Amsterdam and Oxford 1977
8 Evrin P E & Wibell L The serum levels and urinary excretion of β_2 microglobulin in apparently healthy subjects Scand J Clin Lab Invest 29 69 1972
9 Gray G R McKenzie J Smith J S Crean G P & Gillespie G Oral cimetidine in severe duodenal ulceration Lancet 1 4 1977
10 Haggie S J Fermont D C & Wyllie J H Treatment of duodenal ulcer with cimetidine Lancet 1 983 1976
11 Jones R H Rudge C J Bewick M Parsons V & Weston M J Cimetidine prophylaxis against upper gastrointestinal haemorrhage after renal transplantation Br Med J 1 398 1978
12 Peterson P A Evrin P E & Berggård I Differentiation of glomerular tubular and normal proteinuria Determinations of urinary excret on of β_2-microglobulin albumin and total protein J Clin Invest 48 1189 1969
13 Schannon J A The renal excretion of creatinine in man J Clin Invest 14 463 1935
14 Wibell L & Evrin P E Urinary β_2 microglobulin in patients with renal disease—A study during augmented diures Diss 183 Uppsala 1974
15 Wibell L Evrin P E & Berggård I Serum β_2 microglobulin in renal disease Nephron 10 320 1973

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Decreased Renal Plasma Flow during Propranolol Treatment in Essential Hypertension

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ABSTRACT The pharmacodynamic effect of propranolol (80 mg b i d) on the renal and systemic circulation was studied after 1 and 8 months of *treatment in 13 patients with essential hypertension,* using non invasive radioisotope techniques Effective renal plasma flow (ERPF) fell from (mean±S E M) 244±18 to 208+14 after 1 month and to 187±13 ml/min m² after 8 months of treatment Concomitantly cardiac index (CI) fell from 3 24+0 15 to *2 62±0 11 and 2 75±0 10 l/min m , respectively* The coefficient of correlation between the decreases in ERPF and CI was 0 49 Mean arterial blood pressure decreased from 138±5 to 118±5 and 116±4 mmHg respectively Left ventricular work was reduced by 30 2 and 27%, while peripheral resistance was unchanged Total plasma volume was increased from 19 3±0 5 to 20 3±0 6 ml/cm after 1 month but was within the same range as the control values after 8 months of treatment Pulmonary plasma volume was unchanged, indicating that there was no pooling of plasma in the pulmonary circulation The interventricular circulation time was increased from 6 9+0 4 s to 8 4±0 3 s and varied with the change in heart rate It is concluded that the fall in ERPF might be explained by reduced cardiac output in addition to interference with the hemodynamic autoregulation in the kidney

Following i v injection of propranolol a fall in renal blood flow has been observed both in dog (1 13) and in man (14 19)

In a previous study (5) a reduction of effective renal plasma flow (ERPF) was also observed in patients with essential hypertension when blood pressure (BP) was lowered with propranolol (160 mg/day) given therapeutically for 1 month This fall in ERPF was associated with a reduced cardiac output but interference with the hemodynamic autoregulation in the kidney could not be excluded

Considering the very long treatment periods with propranolol in hypertension a further study of the renal and cardiac effects during long term treatment was thought to be of interest ERPF and cardiac output were therefore measured in the present investigation during treatment of essential hypertension with propranolol for 8 months using our established non invasive radioisotope techniques In addition the total and pulmonary plasma volumes and the interventricular circulation times *(IVCT) were measured*

PATIENTS AND METHODS

Thirteen patients 10 males and 3 females 32–61 years of age (mean 51 4 S D ±10 2) were included in the study They had essential hypertension stages I or II according to the WHO classification

The reference values (mean±S D) were as follows height 170 3±8 7 cm weight 82 6±13 4 kg systolic BP 178±19 mmHg diastolic BP 113±9 mmHg cardiac volume (X ray) 440±64 ml/m² and serum creatinine 110±10 µmol/l

Eight patients had never received any antihypertensive treatment five discontinued medication more than 4 weeks before the study started

The investigation was carried out on an out patient basis The patients were received in the laboratory at 8 a m after a light breakfast and no previous exercise The studies were undertaken after 60 min rest in the supine position

After a control measurement of renal and cardiac function the patients were given propranolol (Inderal® ICI) in a dose of 80 mg b i d The studies were repeated after 1 and 8 months of treatment On the days of study the drug was taken 2 hours before the start of measurements

Renography

The individual kidney ^{131}I orthoiodohippuran (^{131}I OIH) clearance ERPF was determined with a renographic technique as described previously (15 16)

Abbreviations ERPF=effective renal plasma flow BP blood pressure IVCT=interventricular circulation time ^{131}I OIH=^{131}I orthoiodohippuran CI=cardiac index CI_{pl}–CI of plasma PCT=pulmonary circulation time PPV pulmonary plasma volume MAP=mean arterial BP LVWI=left ventricular work index TPRI=total peripheral resistance index

Table I *ERPF CI and MAP before (A) and after propranolol treatment for 1 month (B) and 8 months (C)*

Pat no	ERPF (ml/min m²)			CI (l/min m²)			MAP (mmHg)		
	A	B	C	A	B	C	A	B	C
1	201	150	110	2 39	2 25	1 93	136	118	113
2	206	192	174	3 29	2 68	2 21	139	140	131
3	142	126	197	3 65	2 69	2 72	127	122	101
4	245	163	246	3 41	2 99	2 89	139	140	117
5	356	250	201	3 34	2 72	2 92	193	117	126
6	262	199	132	2 47	1 98	2 76	150	157	147
7	262	266	187	3 64	2 45	3 01	128	110	113
8	359	318	264	2 60	3 03	2 68	127	102	119
9	181	196	186	3 72	2 82	2 95	125	93	89
10	302	243	245	3 36	3 36	2 95	133	99	119
11	183	224	139	4 15	2 31	2 72	142	123	115
12	235	187	167	3 24	2 45	3 36	120	119	119
13	269	191	186	2 76	2 31	2 50	131	95	103
Mean	244	208	187	3 24	2 62	2 75	138	118	116
S E M	18	14	13	0 15	0 11	0 10	5	5	4
p		<0 02	<0 01		<0 01	<0 01		<0 01	<0 001

Radiocardiography

Cardiac output was estimated with a radiocardiographic technique employing a gamma camera a Cine 200 computer for rapid sequential uptake and data handling and ^{113m}indium transferrin as the intravascular label as described elsewhere (3) Blood and plasma samples were drawn 10 20 and 30 min after the bolus injection The specific activity of the tracer in these samples allowed for the calculation of tracer concentration at the time of injection by using the equation of a monoexponential function

Renography and radiocardiography were performed in sequence The arterial BP was measured automatically on the arm by Arteriosonde®

Calculation

The basis for ERPF calculation was the uptake by the kidney of ^{131}I OIH 1½–2½ min after the bolus injection (15 16) The ERPF referred to in the present study is the sum of that obtained from each kidney calculated as ml/min m² (4)

The calculation of the radiocardiographic parameters has been described previously (3 5) The blood and plasma volumes were calculated from the dose of ^{113m}In given the specific activity in blood and plasma being corrected to bolus injection time Cardiac index (CI) of plasma (CI_{pl} ml/min m²) was calculated from CI of blood as $CI_{pl} = CI \cdot C_b/C_{pl}$ where C_b is the radioactivity of indium in cpm/ml of the whole blood and C_{pl} the corresponding radioactivity in cpm/ml of plasma at bolus injection time The IVCT for plasma was determined as the time between the peaks of the dilution curves generated for the right and the left ventricles (3)

Mean pulmonary circulation time (PCT) was calculated as 70% of IVCT Pulmonary plasma volume (PPV ml/m²) was calculated by multiplying the CI_{pl}/s with PCT (s) (3)

Mean arterial BP (MAP) was calculated from systolic (SAP) and diastolic (DAP) pressure as MAP = SAP + 2 DAP/3 (mmHg)

Left ventricular work index (LVWI) and total peripheral resistance index (TPRI) were calculated as follows LVWI = [CI (l/min m²) MAP (mmHg) 13 6 g/cm³ l/1000] 0 16 (W/m²)

TPRI = MAP (mmHg) 80/CI (l/min m²) (10⁵ N s m⁻⁵ = dyn s cm⁻⁵ m²)

where 13 6 is the specific gravity of Hg 0 16 a converting factor from kpm/min into watt (W) and 80 a converting factor from mmHg/l/min into dyn s cm⁻⁵

Student s t test for paired observations was used in the statistical calculations

RESULTS

Table I gives the changes in ERPF CI and MAP for the individual patients elicited by propranolol

Compared to the control values ERPF decreased

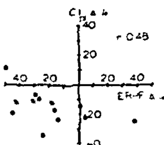


Fig 1 Relationship between changes in ERPF and CI_{pl} after 8 months of propranolol treatment

Table II *Hemodynamic parameters before (A) and after propranolol treatment for 1 month (B) and 8 months (C)*

		Mean	S E M	*p*
Stroke index (ml/beat m²)	A	48	2	
	B	53	2	NS
	C	55	2	<0 01
Heart rate (beats/min)	A	67 3	2 6	
	B	51 5	1 8	<0 001
	C	49 8	1 5	<0 001
LVWI (W/m²)	A	0 96	0 06	
	B	0 67	0 03	<0 001
	C	0 70	0 03	<0 001
TPRI (10⁴ N s m⁻⁵)	A	3 441	237	
	B	3 675	280	NS
	C	3 455	193	NS
IVCT (s)	A	6 9	0 4	
	B	8 4	0 3	<0 001
	C	8 4	0 4	<0 01

NS=$p>0\ 05$

14 8% after 1 month of treatment and 23 4% after 8 months The change from 1 to 8 months was however not statistically significant CI decreased 14 6% in 8 months The relationship between the change in ERPF and CI of plasma is illustrated in Fig 1 The coefficient of correlation between the decrease in CI_{pt} and ERPF was 0 49 MAP fell 15%

Table II lists the changes in stroke index heart rate TPRI LVWI and IVCT As can be seen the stroke index increased as the heart rate decreased LVWI fell 30% after 4 weeks and was unchanged after 8 months of treatment The mean value for TPRI increased after one month but the change was not statistically significant After 8 months TPRI was at the same level as in the control study There was a significant increase in the circulation time for plasma between the right and left ventricle As can be seen from Fig 2 there was a close relationship between the changes in heart rate and IVCT

Table III gives the changes in total plasma and blood volumes in addition to the PPV Total plasma volume showed a transient increase during the first 4 weeks but returned to the control values after 8 months of treatment No change however was observed in blood volume The mean values for PPV were higher during treatment than in the control study but the differences were not statistically significant

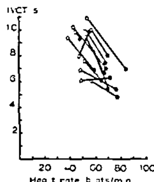


Fig 2 Relationship between heart rate and IVCT in the control study (●) and after 8 months (O) of propranolol treatment

DISCUSSION

The presented data show that propranolol elicited a significant reduction of ERPF during long term treatment and there was no tendency to return to the control levels The observations are consistent with earlier results obtained during i v injection of propranolol (1 5 14 19) and with those obtained during short term treatment with the drug (5) The data are also in agreement with findings of Ibsen and Sederberg Olsen (10) who showed that glomerular filtration rate fell during propranolol administration

The decrease in ERPF cannot be due to reduced cardiac output alone the rather low coefficient of correlation ($r=0\ 48$) between the changes in these parameters can be taken as evidence against it The

Table III *Total plasma volume pulmonary plasma volume and blood volume before (A) and after propranolol treatment for 1 month (B) and 8 months (C)*

		Mean	S E M	*p*
Plasma volume (ml/cm)	A	19 3	0 5	
	B	20 3	0 6	<0 02
	C	19 7	0 7	NS
PPV (ml/m²)	A	146	8	NS
	B	153	7	NS
	C	155	9	NS
Blood volume (ml/cm)	A	32 6	0 8	
	B	33 0	0 7	NS
	C	32 3	1 2	NS

NS=$p>0\ 05$

BP was reduced but still within a range that should be fully compensated for by the renal hemodynamic autoregulation. The fall in ERPF therefore points to an interference with this autoregulation.

Previous clinical studies of kidney function on propranolol treatment have given equivocal results. Kincaid-Smith and Hua (11) and Thomson and Joekes (22) found no deterioration in kidney function during long-term propranolol treatment, while Franzen and Pasternack (6) concluded that the use of β-adrenergic blocking agents in rapidly progressing renal failure can be hazardous. Their description of the studies was, however, brief and incomplete. Warren et al. (23, 24) observed deterioration of kidney function during β-adrenergic blockade in patients with chronic renal failure and hypertension. In addition, Ibsen and Sederberg-Olsen (10) and Drayer et al. (2) found decreased glomerular filtration rate during propranolol administration. Since the patients in this study had normal kidney function, the data do no permit conclusions about the effect of propranolol in patients with deteriorated kidney function.

The reduction in cardiac output and in heart rate and the increase in stroke volume were of the same magnitude as reported by others (8, 12). Left ventricular work was reduced by an effect on cardiac output and BP.

The peripheral resistance showed insignificant changes, in keeping with reports by Hansson et al. (8) and Mookherjee et al. (12). During propranolol treatment Tarazi and Dustan (20) demonstrated that peripheral resistance increased immediately and then gradually returned to the control levels before treatment, indicating a long-term adaptation of peripheral resistance to chronic reduction of cardiac output.

The plasma volume increased to a minor degree during the first 4 weeks of treatment, but fell to the control level during continued treatment. Increased body weight has been observed in acute studies after propranolol administration (2) and has been explained by increased reabsorption of sodium because of increased renal vascular resistance and filtration fraction (14). Only minor changes in plasma volume have previously been observed during long-term treatment in patients with well-compensated ischemic heart disease (9). Propranolol did not elicit a pooling of plasma in the pulmonary circulation since PPV remained unchanged.

The velocity of plasma flow was considerably decreased since the circulation time was increased. This implies a lower kinetic energy of the flowing blood and therefore also a reduced kinetic work of the heart.

ACKNOWLEDGEMENT

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REFERENCES

1. Carriere, S.: Effect of norepinephrine, isoproterenol and adrenergic blockers upon the intrarenal distribution of blood flow. Can J Physiol Pharmacol 47: 199, 1969.
2. Drayer, J. I. M., Kloppenborg, P. W. C., Festen, J., van 't Laar, A. & Benraad, T. J.: Intrapatient comparison of treatment with chlorthalidone, spironolactone and propranolol in normoreninemic essential hypertension. Am J Cardiol 36: 716, 1975.
3. Falch, D. K. & Norman, N.: Evaluation of a computerized technique for determination of cardiac output and central circulation times using gamma camera and 113mindium. Scand J Clin Lab Invest 34: 207, 1974.
4. Falch, D. K. & Norman, N.: The cardiac response to a small i.v. dose of dihydralazine, a safe drug for diagnostic tests? Acta Med Scand 203: 433, 1978.
5. Falch, D. K., Norman, N. & Ødegaard, A. E.: Renal plasma flow and cardiac output during hydralazine and propranolol treatment in essential hypertension. Scand J Clin Lab Invest 38: 143, 1978.
6. Franzen, P. & Pasternack, A.: Propranolol in renal failure. Br Med J 3: 763, 1972.
7. Frohlich, E. D., Tarazi, R. C., Dustan, H. P. & Page, I. H.: The paradox of beta-adrenergic blockade in hypertension. Circulation 37: 417, 1968.
8. Hansson, L., Zweifler, A. J., Julius, S. & Hunyor, S. N.: Hemodynamic effects of acute and prolonged β-adrenergic blockade in essential hypertension. Acta Med Scand 196: 27, 1974.
9. Hesse, B., Bollerup, A. C. & Olesen, K. H.: The influence of beta-blocking agents on plasma volume and extracellular volume in ischaemic heart disease. Scand J Clin Lab Invest 34: 215, 1974.
10. Ibsen, H. & Sederberg-Olsen, P.: Changes in glomerular filtration rate during long-term treatment with propranolol in patients with arterial hypertension. Clin Sci Mol Med 44: 129, 1973.
11. Kincaid-Smith, P. & Hua, A. S. P.: Beta-adrenergic blocking agents in renal failure. Br Med J 3: 520, 1974.
12. Mookherjee, S., Eich, R. H., Obeid, A. I. & Smulyan, H.: Hemodynamic and plasma renin effects of propranolol in essential hypertension. Arch Intern Med 137: 290, 1977.
13. Nayer, W. G., McInnes, I., Swann, J. B., Carson, V. & Lowe, T.: Effect of propranolol, a beta-adrenergic antagonist, on blood flow in the coronary and other vascular fields. Am Heart J 73: 207, 1976.

14 Nies A S McNeil J S & Schrier R W Mechanism of increased sodium reabsorption during propranolol administration Circulation 46 596 1971
15 Norman N Effective plasma flow of the individual kidney Determination on the basis of the 131I hippuran renogram Scand J Clin Lab Invest 30 395 1972
16 Norman N Sundsfjord J A & Sturis G Effective renal plasma flow (ERPF) of the individual kidney and renal venous renin activity (RVRA) determined simultaneously before and after the administration of dihydralazine in renovascular hypertension Scand J Clin Invest 35 219 1975
17 Sederberg-Olsen P & Ibsen H Plasma volume and extracellular fluid volume during long term treatment with propranolol in essential hypertension Clin Sci Mol Med 43 165 1972
18 Smith E C Dhar S K & Freedman P Propranolol in the management of hypertension in a long term dialysis program JAMA 229 1777 1974
19 Sullivan J M Adams D F & Hollenberg N K β adrenergic blockade in essential hypertension Reduced renin release despite renal vasoconstriction Circ Res 39 532 1976
20 Tarazi R C & Dustan H P Beta adrenergic blockade in hypertension Practical and theoretical implications on long term hemodynamic variations Am J Cardiol 29 633 1972
21 Tarazi R C Frohlich E D & Dustan H P Plasma volume changes with long term beta adrenergic blockade Am Heart J 82 770 1971
22 Thomson F D & Joekes A M Beta blockade in the presence of renal disease and hypertension Br Med J 2 555 1974
23 Warren D J Beta adrenergic receptor blockade and renal function Am Heart J 91 265 1976
24 Warren D J Swainson C P & Wright N Deterioration in renal function after beta blockade in patients with chronic renal failure and hypertension Br Med J 2 193 1974

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Treatment of Refractory Anemias with Methenolone

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ABSTRACT A therapeutic trial with methenolone (Primobolan®) in 19 consecutive patients with different types of refractory anemia is reported The remission frequencies were pancytopenia 3/6, bicytopenia 2/4, refractory anemia with hyperplastic marrow 1/5 myelofibrosis 1/4 There was no obvious prolongation of survival in the patients responding Side effects were negligible

Key words Anabolic steroids methenolone pancytopenia refractory anemias myelofibrosis

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Since the introduction of androgenic anabolic steroids in the therapy of aplastic anemias by Coletta et al (2) and Seip (13) in 1961 the majority of reports concern oxymetholone (11 12) The effects of this drug in different anemias are quite well documented its most serious drawback is its liver toxicity (11 12) Recently liver tumours have been reported after treatment with oxymetholone (1)

The androgenic anabolic steroids nandrolone and methenolone seem to have similar antianemic properties but a lower liver toxicity than oxymetholone (11 12) Reports on the use of these drugs are scanty especially with respect to long term effects Nandrolone has to be injected methenolone may be taken orally Therefore the author found it worth while to collect further material on the use of methenolone especially with respect to long term survival and toxicity

PATIENTS AND METHODS

Nineteen consecutive patients were studied The diagnoses were based on repeated determinations of peripheral blood values with clinical routine methods and on at least one bone marrow biopsy where smears as well as sections of coagulated bone marrow were studied The peripheral blood values were determined at short intervals before during and after treatment Methenolone (Primobolan®) 25 mg (Schering Nordiska AB Nacka Sweden) was given initially in doses between 100 and 300 mg/day the maintenance dose was sometimes lower

The effect of the treatment was classified as complete remission when normalization of Hb leucocyte and thrombocyte values was achieved Partial remission was defined as a permanent increase in Hb values by more than 10%

Before starting the treatment and once a month during treatment bilirubin ASAT ALAT and alkaline phosphatases in serum were analysed by routine methods

RESULTS AND DISCUSSION

Data on the patients are listed in Tables I–III

Pancytopenia (patients 1–6)

This diagnosis was based on the demonstration of a hypocellular bone marrow and subnormal Hb leucocyte and thrombocyte values prevailing between four months and three years

This group of patients included two women and four men and consisted of so-called idiopathic pancytopenias where no causative agents could be found except in one woman in whom lues congenita and cachexia may have played a causative role One patient went into complete and two into partial remission The effect of therapy started to become measurable after 4–7 months In none of the responding patients could treatment be discontinued The non responders have all died by now Only one of the responders is still alive

When using androgenic anabolic steroids the reported remission frequencies lie around 50% (7 9 11 12) In the small series reported here the results are of a similar magnitude

The 50% survival for patients with aplastic anemia is reported to lie between a few months for untreated (5) and treated patients (10) and up to more than three years for patients treated with oxymetholone (7) The great differences in survival reported seem primarily to be caused by the incomparableness of the patient groups and the

Table 1 *Clinical data on the patients*

Pat no	Age (y)	Sex	Diagnosis	Duration of disease before methenolone	Previous treatment	Blood values before methenolone		
						Hb (g/l)	WBC ($\times 10^9$/l)	Thrombocytes ($\times 10^9$/l)
1	48	♂	Idiopathic pancytopenia	4 mo	Prednisolone testosterone folic acid B_{12}	45	2 4	4
2	66	♂	Idiopathic pancytopenia	1 y	Prednisolone pyridoxine testosterone B_{12} transfusions	52	1 5	[illegible]
3	88	♀	Idiopathic pancytopenia	>6 mo	B_{12} Fe prednisolone transfusions	100	1 2	38
4	77	♂	Idiopathic pancytopenia	3 y	Prednisolone pyridoxine transfusions B_{12} folic acid	90	8 5	30
5	71	♂	Idiopathic pancytopenia	1 y	Transfusions 3 l/mo	60	2 2	37
6	46	♀	Pancytopenia (lues congenita cachexia)	>1 y	Prednisolone pyridoxine folic acid transfusions	91	1 3	78
7	82	♂	Idiopathic bicytopenia	2 y	Folic acid pyridoxine	51	3 9	191
8	50	♀	Bicytopenia (rheumatoid arthritis normal spleen)	7 mo	Prednyliden pyridoxine folic acid blood and thrombocyte transfusions	60	8 0	5
9	73	♀	Bicytopenia (CML overdose of cytostatics)	2 mo	Busulfan cyklophosphamide prednisolone folic acid vitamin B B_{12} transfusions	135	0 6	43
10	69	♀	Granulocytopenia (oxyphenbutazone)	1 mo	Prednisolone	123	0 67	190
11	62	♂	Refractory anemia (hyperplastic bone marrow) (smouldering leukemia—acute myeloblastic leukemia)	2 mo	Transfusions	86	4 1	30
[illegible]	71	♀	Refractory anemia (hyperplastic bone marrow)	10 y	Folic acid pyridoxine B_{12} prednisolone nandrolone-decanoate transfusions testosterone	75	4 0	300
13	73	♂	Refractory anemia (hyperplastic bone marrow) (smouldering leukemia—promyelocyte leukemia)	2 y	B_{12} Fe folic acid pyridoxine transfusions	65	6 4	124
14	61	♂	Refractory anemia (hyperplastic bone marrow) (smouldering leukemia)	3 mo	Folic acid pyridoxine	105	3 7	117
15	75	♂	Refractory anemia	1 y	Fe B_{12} folic acid prednisolone pyridoxine transfusions	70	5 0	260
16	69	♂	Myelofibrosis (preleukemia?)	1 y	Vitamin B B_{12} pyridoxine prednisolone	76	5 9	77
17	67	♀	Myelofibrosis	6 y	Fe pyridoxine folic acid	103	4 8	257
18	79	♂	Myelofibrosis	18 mo	Fe	93	6 7	62
19	80	♂	Myelofibrosis	6 mo		92	13 3	27

Table II *Duration of treatment blood values after treatment and side effects*

Pat no	Daily methenolone dose (mg)	Duration of treatment (mo)	Simultaneous treatment	Duration of treatment until effect (mo)	Blood values after methenolone Hb (g/l)	WBC ($\times 10^9$/l)	Thrombocytes ($\times 10^9$/l)	Side-effects
1	300	4	Prednisolone erythrocyte and thrombocyte *transfusions*		47	3 0–2 0	2–8	
2	100	9	As before		As before			
3	200 250	2 (pause 8 mo) 4	Prednisolone		75	1 0	20	Hirsutism coarseness of voice increase of bilirubin + alkaline phosphatases
4	250 300	3 4	Pyridoxine prednisolone no transfusions	4	110	1 1	40	
5	300 150	6 6	Prednisolone no transfusions	6	116	4 6	98	Slight increase of transaminases acne (?)
6	100 150 Maintenance dose 50	4 10	Pyridoxine folic acid	7	146	3 8	231	Acne hypertrichosis
7	100	2	Prednisolone	2	112	4 8	102	
8	300 250	1 12	Folic acid pyridoxine prednyliden	3	159	6 5	65	Coarseness of voice hirsutism acne
9	250	2	Prednisolone B_{12} B vitamins folic acid transfusions		112	0 6	17	
10	300	2	Prednisolone		118	0 36	242	Transient increase of transaminases 47/90 later 12/24 (n=40/40)
11	250 300	3 1	Transfusions		98	9 8	30	
12	100	28	Folic acid pyridoxine B_{12} prednisolone nandrolone-decanoate more transfusions		60	3 0	200	Coarseness of voice loss of hair hirsutism less than with testosterone
13	100	6	Prednisolone transfusions		87	3 8	32	
14	100	8		2	122	5 6	170	
15	300	8	Folic acid B_{12} transfusions		60	3 3	120	GPT 47 GOT normal (n=40)
16	200	3	Transfusions		87	15 5	22	
17	150 50	4 2	Folic acid	4	128	4 8	202	Coarseness of voice hirsutism slight oedema Normalization except changes of voice after cessation of therapy
18	150 200	5 8			105	8 1	181	
19	150	6	B_{12} folic acid Fe		As before			

Table III *Effect of treatment and survival*

PR = partial remission CR = complete remission

Pat no	Effect of treatment	Total survival	Treatment survival	Comments
1		8 mo	4 mo	Died in bleeding
2		6 y	5 y	Died in pneumonia
3		3 y	3 5 y	Died in bleeding treatment discontinued because of side-effects
4	PR	5 y	2 y	Died in pancytopenia
5	PR	3 y	2 y	Alive Recurrence 6 mo after cessation of methenolone effect again when reinstituted
6	CR	30 mo	20 mo	Died in pneumonia
7	PR	32 mo	8 mo	Died in pneumonia Relapse 3 mo after discontinuation of methenolone
8	PR	6 y	5 y	Alive 9 mo after remission change to oxymetholone 100 mg daily Oxymetholone stopped after 1 y because of liver toxicity Hb 135 thrombocytes 100 WBC 2 2
9		4 mo	2 mo	Died in pericarditis
10		3 mo	2 mo	Died in myocardial infarction
11		6 mo	4 mo	Died in cerebral bleeding
12		12 y 4 mo	28 mo	Died in myocardial infarction
13		30 mo	6 mo	Died in cardiac insufficiency
14	CR	18 mo	15 mo	Died in myocardial infarction 7 mo after stopping treatment No effect on reinstitution
15		3 y	2 y	Died in cardiac insufficiency
16		15 mo	3 mo	Died in cerebral bleeding
17	CR	12 y	6 y	Alive Normal values 1 y after cessation of treatment Relapse after 2 further y
18	PR	30 mo	13 mo	Died in cardiac insufficiency
19				Died in cardiac failure + myelofibrosis

teadily increasing quality of supporting care given to them The present patients show considerable differences in survival without an obvious difference between those who responded to treatment and those who did not These findings show that pancytopenia still has a very serious prognosis which is not significantly affected by methenolone This makes bone marrow transplantation an attractive alternative in suitable cases

Bicytopenia and granulocytopenia
(patients 7–10)

In these cases the bone marrow was hypoplastic In patient 7 no precipitating agent could be found In the other three cases cytostatics or antirheumatic drugs seem to have caused the cytopenias In the patient with idiopathic bicytopenia a complete remission was achieved one of the patients taking antirheumatics went into partial remission Only one of these patients survived the prognosis thus being not much improved by methenolone

Refractory anemia
(patients 11–15)

All patients included in this group had hypercellular bone marrows with increased amounts of iron and sideroblasts Leucocyte and thrombocyte values were affected differently

This group comprised four men and one woman The treatment was successful in only one male patient He went into complete remission after two months of treatment He died two years later in a myocardial infarction A few weeks before his death the bone marrow showed signs of smouldering leukemia Two further patients in this group developed smouldering leukemia The patients survived between six months and 12 years

Treatment with androgenic anabolic steroids has been reported only sporadically in such cases (7 11 12 14) and the results have been disappointing The feasibility of treating patients running such a great risk of developing leukemia with androgenic anabolic steroids has been questioned (4)

Myelofibrosis (patients 16–19)

Four patients were treated three men and one woman complete remission was achieved in the only woman in this group After six months of treat ment remission was maintained for 2 5 years Thereafter moderate anemia has slowly reappeared the patient has so far refused reinstitution of therapy because of its virilizing properties In another case temporary partial remission was achieved All patients except the woman are dead

Sanchez Medal (11) reviewed the results obtained in more than 50 such cases androgenic anabolic steroids were effective in about half the cases Hast et al (6) saw an effect in eight out of 11 cases treated with oxymetholone

Side effects

The most frequent side effect of methenolone treatment was virilization of female patients in the form of hirsutism and coarseness of the voice in six of the nine women treated In no instance did treatment have to be discontinued for this reason but one woman (no 17) refused to resume treat ment after it had been withdrawn for a considerable time because of remission of anemia

Signs of liver toxicity were observed in only four of the 19 patients treated They consisted of slight elevations of transaminase values in three and of a more marked increase in bilirubin and alkaline phosphatase values in one patient Treatment had to be discontinued in the latter case only

The results of the present study are in congruence with the observations of others on the much lower liver toxicity of androgenic anabolic steroids without alkylation in the 17 position (11 12)

REFERENCES

1 Bagheri S A & Boyer J L Peliosis hepatis associated with androgenic anabolic steroid therapy Ann Intern Med 81 610 1974

2 Coletta A Esposito L & Palomby L Su alcuni recenti orientamenti terapeutici in tema di pancitopenia ipoplastica Testosterone e steroidi anabolici Pediatria 69 413 1961

3 Davies M Muckle T J & Casdells Smith A Oxymetholone in the treatment of anemia in chronic renal failure Br J Urol 44 387 1972

4 Ginsburg A D Oxymetholone and hematologic disease Ann Intern Med 79 914 1973

5 Gordon Smith E C Kiley N & Lewis S M Aplastic anaemia-classification and management Br J Haematol 25 277 1973

6 Hast R Jameson S Killander A Lundh B Reizenstein P Skårberg K O Uden A M & Wadman B Oxymetholone treatment in myelofibrosis Blut In press 1978

7 Hast R Skårberg K O Engstedt L Jameson S Killander A Lundh B Reizenstein P Uden A M & Wadman B Oxymetholone treatment in aregenerative anaemia II Remission and survival—a prospective study Scand J Haematol 16 90 1976

8 Koch K M Patyna W D Shaldon S & Werner E Anemia of the regular hemodialysis patient and its treatment Nephron 12 405 1974

9 Mori M Chiba S Suzuki S Kosaka K & Takaku F Studies on the mechanism of erythropoietic effect of methenolone in vitro Erythropoiesis In Proceedings of the fourth international conference on erythropoiesis 1974 (ed K Nakao J W Fisher & F Takaku) pp 85–90 University Park Press Baltimore 1975

10 Sacks P Gale D Bothwell T H & Stevens K Oxymetholone therapy in aplastic and other refractory anaemias S Afr Med J 46 1607 1972

11 Sanchez Medal L Therapie hamatologischer Erkrankungen mit Androgenen Munch Med Wochenschr 25 960 1971

12 Sanchez Medal L Gomez Leal A Duarte L & Rico M G Anabolic androgenic steroids in the treatment of acquired aplastic anemia Blood 34 283 1969

13 Seip M Aplastic anemia treated with anabolic steroids and corticosteroids Acta Paediatr Scand 50 561 1961

14 Skårberg K O Engstedt L Jameson S Killander A Lundh B Pers B Reizenstein P Uden A M & Wadman B Oxymetholone treatment in hypoproliferative anaemia. I Frequency of response Acta Haematol 49 321 1973

15 Udupa K B & Reissmann K R Acceleration of granulopoietic recovery by androgenic steroids in mice made neutropenic by cytotoxic drugs Cancer Res 34 2517 1974

16 Williams J S Stein J H & Ferris T F Nandrolone decanoate therapy for patients receiving hemodialysis Arch Intern Med 134 289 1974

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Autoantibodies and Serum Immunoglobulins in Chronic Liver Diseases

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ABSTRACT In a prospective consecutive study, 68 patients with various liver diseases and 67 control persons were examined for the occurrence of smooth muscle antibodies (SMA), antinuclear antibodies (ANA) and mitochondrial antibodies (MTA) of IgG IgA and IgM class A determination of serum immunoglobulins (S IgG, S IgA and S IgM) was also performed IgG SMA in titres of >80 occurred in 8 of 12 patients (67%) with hepatitis B antigen (HBag) negative chronic active liver disease (CALD) and not in other diseases Apart from one patient with primary biliary cirrhosis (PBC) IgG ANA in titres of >40 were likewise detected only in HBag negative CALD (33%) The titres of IgG SMA and IgG ANA varied analogously with the biochemical liver parametres There was a mutual exclusion between HBag and IgG SMA/ ANA in titres of >20, while IgM SMA occurred in titres of 80 in two patients with HBag positive CALD The incidence and titres of IgM SMA and ANA were not higher than in the controls IgA SMA and ANA were detected only sporadically The MTA demonstrated were of IgG class and titres of >40 were found only in patients with PBC (4 of 5) Some of the patients in all groups had an increased concentration of one or more of the serum immunoglobulins S-IgG levels were found to be significantly higher in CALD than in the other groups

Key words Chronic liver disease smooth muscle antibodies antinuclear antibodies mitochondrial antibodies serum immunoglobulins

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Several studies have dealt with the occurrence of smooth muscle antibodies (SMA) antinuclear antibodies (ANA) and mitochondrial antibodies (MTA) in various liver diseases (4 6 9 11 12 13 16 25) SMA is demonstrated with a high incidence in acute (4 13) and chronic hepatitis (9 11 12) occurring in the highest titres in chronic active/aggressive hepatitis (10) MTA is at least in high titres considered to be a reliable diagnostic marker for primary biliary cirrhosis (10)

The aim of this investigation was to study the correlation between autoantibodies together with serum immunoglobulin values and morphological and biochemical changes in various chronic liver diseases in order to estimate the diagnostic and prognostic value of the determination of these immunological parameters

PATIENTS AND METHODS

The patient group consisted of 68 consecutive persons who during a 28-month period were hospitalized for the estimation of a liver disease On the basis of liver biopsy anamnesis and biochemical changes the patients were classified into the following groups Patients in whom no biopsy was taken were excluded

Chronic active liver disease (CALD)

This group comprised 16 patients with chronic liver disease with current inflammatory activity Fourteen had liver bioptic changes corresponding to chronic aggressive hepatitis (8) and seven of these had cirrhosis as well One patient had subacute hepatitis with bridging (5) and one subacute hepatitis with multilobular necrosis (5) and cirrhosis For final placing in the group a course of at least 10 weeks without recovery was required (5)

Chronic inactive liver disease (CILD)

This group comprised 21 patients with chronic liver disease with no present inflammatory activity Fourteen had bioptic changes corresponding to chronic persistent hepatitis (8) and seven had inactive cirrhosis Eight pa

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Abbreviations SMA=smooth muscle antibodies ANA=antinuclear antibodies MTA mitochondrial antibodies CALD=chronic active liver disease CILD=chronic inactive liver disease PBC=primary biliary cirrhosis MISC=miscellaneous liver disease S-GOT=serum glutamic oxaloacetic transaminase S-Ig=serum immunoglobulin HBag=hepatitis B antigen

Table I *Clinical data on the patients and controls*

	Sex		Age (y)		Duration of symptoms (mo)		Steroid treatment (no of pats)		
	♂	♀	Range	Median	Range	Median	At start	During the study	Investigated more than once
CALD (N=16)	5	11	22-72	54	1-96	5	2	10	14
CILD (N=21)	8	13	18-73	52	4-84	29	11	4	18
PBC (N=5)	0	5	46-72	64	2-46	2	1	2	3
MISC (N=26)	16	10	19-79	56	<1-58	3	0	–	–
Controls (N=67)	29	38	25-76	55	–	–	0	–	–

tients all on treatment with prednisone had previously had chronic aggressive hepatitis These eight patients did not differ histologically or biochemically from the other CILD patients

Primary biliary cirrhosis (PBC)

Five patients had PBC based on the coexistence of the following criteria (*a*) laboratory indication of chronic cholestasis (*b*) normal extrahepatic biliary tract shown by X ray (*c*) liver biopsic changes consistent with PBC (22)

Miscellaneous liver diseases (MISC)

This group comprised 26 patients Sixteen had alcoholic liver diseases (11 cirrhosis 3 steatosis 2 alcoholic hepatitis) based on liver histology (23) together with an anamnestic alcohol consumption of at least 5 daily drinks (about 60 g alcohol daily) for at least 5 years Six patients had extrahepatic biliary obstruction verified by operation. Three had liver metastases and one had constitutional perbilirubinaemia.

ne control group consisted of 67 persons 7 members the hospital staff and 60 hospitalized patients without anamnestic or biochemical indication of liver diseases autoimmune infectious or neoplastic diseases Liver biopsy was not performed. The controls were matched with the patients for sex and age

Liver histology Liver biopsy a m Menghini (20) was performed in all patients at the beginning of the investigation The biopsy was evaluated at the Department of Pathology Randers City Hospital

Biochemical liver tests Serum bilirubin serum glutamic oxaloacetic transaminase (S-GOT) serum alkaline phosphatase serum albumin and serum gammaglobulin were determined by routine methods at the Department of Clinical Chemistry Randers City Hospital

Hepatitis B associated antigen (HBag) was detected by radioimmunoassay (Austria II)

Autoantibodies SMA ANA and MTA were detected by means of the indirect immunofluorescence method (1 3) As antigens were used 4 μm sections of rat kidney and rat stomach. All sera were investigated for antibodies of the IgG IgA and IgM classes by means of monospecific fluorescein isothiocyanate (FITC)-conjugated antihuman globulins (Wellcome Research Laboratories England and

Table II *Occurrence of SMA ANA and MTA of IgG IgA and IgM class at the initial investigation*

	SMA			ANA			MTA		
	IgG	IgA	IgM	IgG	IgA	IgM	IgG	IgA	IgM
CALD (N=16)									
N pos	12	1	4	8	4	3	0	0	0
Titres	10-1 280	10	10-80	20-5 120	20-40	10-40			
CILD (N=21)									
N pos	10	0	0	2	1	4	1	0	0
Titres	10-80			40	10	10-160	40		
PBC (N=5)									
N pos	2	1	2	1	1	2	4	0	0
Titres	10-20	10	10-80	2 560	80	20-640	160-1 280		
MISC (N=26)									
N pos	1	0	2	1	1	3	0	0	0
Titres	20		10	20	10	10-80			
Controls (N=67)									
N pos.	4	0	16	1	0	16	0	0	0
Titres	10		10-40	10		10-40			

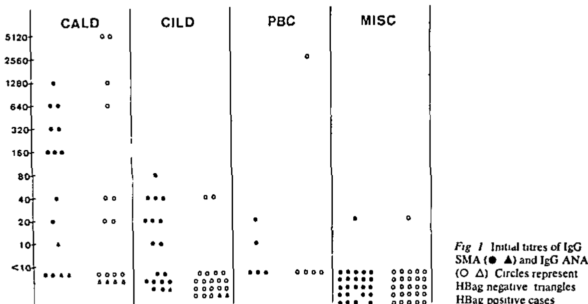


Fig 1 Initial titres of IgG SMA (● ▲) and IgG ANA (○ △) Circles represent HBag negative triangles HBag positive cases

Behringwerke Germany) The molar fluorescein protein ratios were 2 0–4 0 The conjugates were used in a dilution corresponding to an antibody content of 1 4 U/ml All sera were initially tested in a dilution of 1 10 and positive sera were titrated in doubling dilutions

Serum immunoglobulin (S Ig) determinations S IgG S IgA and S-IgM were measured by the method of radial immunodiffusion (19) using immunodiffusion plates standards and control sera from Behringwerke Germany The variation coefficient was for S IgG 9 6% for S IgA 8 5% and for S IgM 5 4% based on 26 31 and 26 double determinations respectively

Patients with CALD CILD and PBC had repeated determinations of the immunological and biochemical parameters every 3 months during the whole observation period which covers 3–28 months Patients with MISC and controls were investigated once only Most patients with CALD were treated with prednisone or a combination of prednisone and azathioprine if prednisone alone did not lead to remission The other patients did not receive immunosuppressive treatment except some patients with CILD who were on treatment with prednisone at the start of the study

RESULTS

Table I gives the age and sex of the patients and controls the duration of liver disease before the study and the number of patients treated with prednisone at the beginning of and during the study

The occurrence of SMA ANA and MTA at the first investigation is shown in Table II SMA and ANA of all three Ig classes were demonstrated and some sera contained antibodies with the same specificity in several Ig classes IgA SMA and IgA ANA were in eight of nine cases found together with IgG antibodies with the same specificity In patients with MISC and in controls SMA and ANA were mainly of the IgM class While the incidence of IgM antibodies was just as high in controls as in patients IgG SMA was found significantly more often in patients with CALD (75%) and CILD (48%) than in controls (6%) ($p<10^{-7}$ and $p<10^{-4}$ respectively) as well as in patients with MISC (4%) ($p<10^{-5}$ and $p<0.01$ respectively) and IgG ANA occurred with a significantly increased incidence in patients with CALD (50%) compared to controls (1%) patients with MISC (4%) and patients with CILD (10%) ($p<10^{-5}$ $p<0.01$ and $p<0.02$ respectively)

Six patients four with CALD and two with CILD had HBag in serum None of these had IgG ANA and the IgG SMA titres were below 40 (Fig 1) while IgM SMA titres of 80 were found in two patients with HBag positive CALD Fig 1 shows the initial titres of IgG SMA and ANA IgG SMA in titres above 80 were only found in patients with HBag negative CALD here in 8 of 12 (67%) Ten of 12 patients with HBag negative CALD had IgG SMA and/or IgG ANA in titres of ≥160 These titres were not correlated to the biochemical liver parameters or to the duration of the

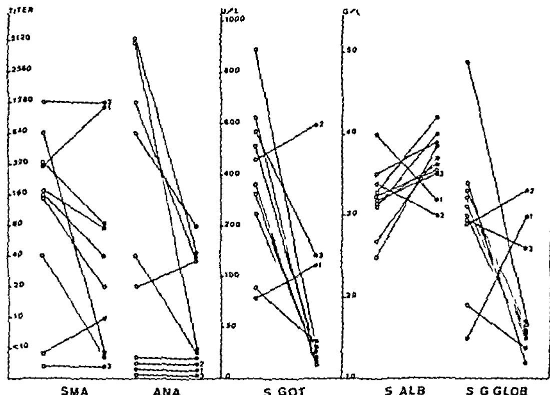


Fig 2 Simultaneous variation of IgG SMA IgG ANA S-GOT serum albumin (*S ALB*) and serum gammaglobulin (*S-G GLOB*) in 10 patients with HBag negative CALD Values at start and end of the investigation are indicated Nine patients were treated with prednisone or a combination of prednisone and azathioprine Cases with continuous activity are numbered ● = Immunosuppressive treatment O = no treatment

disease Among the ten CILD patients with IgG SMA six had previously had chronic aggressive hepatitis The four CILD patients with IgG SMA in titres of ≥40 had all previously suffered from chronic aggressive hepatitis The occurrence of autoantibodies was not correlated to the presence of cirrhosis in CALD or CILD patients

During the course of HBag negative CALD the titres of IgG SMA and IgG ANA varied in parallel with the biochemical liver parameters (Fig 2) Data from two patients who were only investigated for 3 months are not included in the figure Serum bilirubin varied like S-GOT Seven of the ten patients mentioned remitted during the observation period These seven had all initially IgG SMA and/or IgG ANA titres of ≥160 and in remission the titres fell to ≤80 In three patients the disease was persistently active and in two of these IgG SMA titres remained high These two patients were ANA negative and the third patient had neither ANA nor SMA

After discontinuation of immunosuppressive treatment three patients with CILD developed progressive biochemical changes indicating a recurrence of CALD In two of these the IgG SMA titres increased from 80 to 640 and from 20 to 80 respectively In the third the IgG ANA titre rose from 40 to 160 In none of the other CILD patients did the biochemical parameters or the autoantibody titres change during the study

Fig 3 shows the initial S Ig concentrations Within all groups some patients had increased concentration of one or more S-Ig but the ranges were wide with considerable overlapping between the groups S-IgG values were significantly higher in patients with CALD than in the other groups ($p<0.01$ Mann Whitney rank sum test) In the seven patients with HBag negative CALD who remitted during the study S-IgG fell significantly from 32 4 to 14 8 g/l (median values $p<0.01$ Mann Whitney test) S-IgA and S IgM did not change significantly In the MISC group elevated S-Ig values were only found among patients with alcoholic cirrhosis in whom especially S-IgA levels

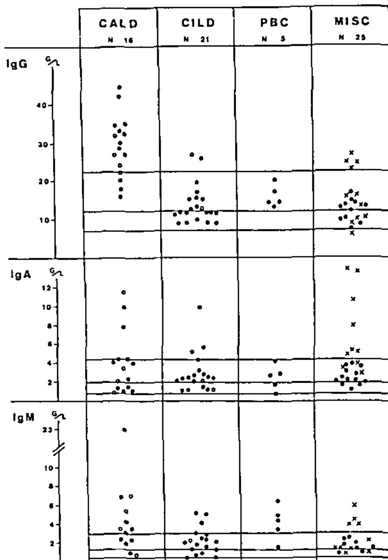


Fig 3 Initial concentrations of S IgG S IgA and S IgM Horizontal lines indicate the median and the 2½ and 97½ percentiles for the controls
●=HBag negative cases
O=HBag positive cases
×=alcoholic cirrhosis

were increased In HBag negative CALD a correlation was demonstrated between IgG ANA and S IgG levels (0 02>p>0 01 Spearman s rank correlation test with correction for tied pairs) Apart from this no correlation was found between autoantibody titres and S Ig values

DISCUSSION

The high incidence of SMA and ANA in patients with chronic aggressive hepatitis with or without cirrhosis (CALD) is in agreement with previous findings (9 11 12 25) The antibodies were mainly of IgG class and the titres frequently exceeded 80 The incidence of IgM SMA and ANA did not differ from the control values and the IgA antibodies occurred only sporadically and usually in sera with IgG antibodies of the same specificity In patients with chronic persistent hepatitis and inactive cirrhosis (CILD) IgG SMA were also detected with increased frequency but the titres were lower than in CALD patients and did not exceed 80 A mutual exclusion between HBag and SMA/ANA in chronic hepatitis has been found in some investigations (9 25) but not in others (12 26) We found a mutual exclusion between HBag and SMA/ANA of the IgG class in titres above 20 whereas IgM SMA were detected in titres of 80 in HBag positive CALD This may explain why the presence of HBag and

autoantibodies were not mutually exclusive in investigations where antibodies of different Ig classes were not studied separately (12 26)

As previously demonstrated (11) the occurrence of SMA and ANA in patients with alcoholic liver diseases and extrahepatic biliary obstruction did not differ from that in the controls However in another study (6) and increased incidence of SMA was found in alcoholic cirrhosis but the titres were below 80 SMA also occur in acute hepatitis (4 13) in infectious mononucleosis (15) and in cytomegalovirus infection (2) but in these diseases SMA are most often of the IgM class and the titres are seldom above 80

Thus IgG SMA in titres of ≥160 seem to be specific for HBag negative CALD and they may therefore be of differential diagnostic value in liver diseases IgG ANA on the other hand may be a less reliable indicator of CALD occurring in high titres in a variety of diseases (10) In CILD patients treated with prednisone IgG SMA titres of ≥40 may be suspect of immunosuppressed CALD (5)

Among the patients with HBag negative CALD no correlation could be found between the titres of IgG SMA and IgG ANA and the biochemical liver parameters but in the individual course the titres of IgG SMA and ANA varied analogously with the biochemical liver tests with decrease of the titres o ≤80 in cases with remission This finding is in cordance with some investigations (18 24) and in ontrast to others (21) In HBag negative chronic hepatitis and cirrhosis the titres of IgG SMA and ANA seemed to be related to the inflammatory activity presumably reflecting another aspect of the activity than the biochemical liver tests These antibodies might therefore possibly be used together with the biochemical tests to follow the immunosuppressive treatment of CALD

No prognostic importance could be attached to SMA and ANA in the present investigation This may be due to the small number of patients or to the short observation period However it should be mentioned that among patients with HBag negative CALD six out of seven who remitted on immunosuppressive treatment had IgG ANA while three who did not remit were ANA negative

The MTA demonstrated were of the IgG class and titres above 40 were found only in patients with PBC This is in agreement with studies of larger groups of patients (11 16) and MTA might therefore be used to differentiate PBC from extrahepatic biliary obstruction Lam et al (17) however found MTA in high titres in patients with prolonged extrahepatic biliary obstruction and they observed that MTA disappeared after surgical decompression We could not demonstrate MTA in patients with extrahepatic biliary obstruction but we only studied a few patients all of whom had a duration of disease of less than 3 months

Increased S IgG concentrations occurred most frequently in patients with CALD (12 14) and very high values were found only in these patients Increased S IgM have previously been found to characterize PBC as opposed to extrahepatic biliary obstruction (7) and our results are in accordance with this Increased S IgA values were especially found in patients with alcoholic cirrhosis (6 14) However as demonstrated by others (12 14) there was a considerable overlapping of the different S Ig values in the various patient groups studied This renders the determinations of S Ig concentrations less valuable as differential diagnostics aids in liver diseases

As a conclusion it can be said that the demonstration of SMA ANA and MTA of IgG class may be of diagnostic value in liver diseases while the determination of the corresponding IgA and IgM antibodies does not offer further information In the antoantibody positive cases the titres of IgG SMA and IgG ANA seem to be related to the inflammatory activity The antibodies may therefore possibly be used as parameters to follow the immunosuppressive treatment of CALD but in order to determine this more long term prospective investigations are necessary

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REFERENCES

1 Andersen P Indirect immunofluorescence studies of smooth muscle antibodies Acta Pathol Microbiol Scand (B) 82 577 1974
2 Andersen P & Andersen H K Smooth muscle antibodies and other tissue antibodies in cytomegalovirus infection Clin Exp Immunol 22 22 1975
3 Andersen P & Beutner E H Defined immunofluorescence studies of cytoplasmic antibodies Int Arch Allergy 43 780 1972
4 Andersen P Thestrup-Pedersen K & Ladefoged K Studies of smooth muscle antibodies in acute hepatitis Acta Pathol Microbiol Scand (C) 84 365 1976

5 Baggenstoss A H Soloway R D Summerskill W H J Elveback L R & Schoenfield L J Chronic active liver disease Hum Pathol 3 183 1972
6 Bailey R J Krasner N Eddleston A L W Williams R Tee D E H Doniach D Kennedy L A & Bachelor J R Histocompatibility antigens autoantibodies and immunoglobulins in alcoholic liver disease Br Med J 2 727 1976
7 Bevan G Baldus W P & Gleich G J Serum immunoglobulin levels in cholestasis Gastroenterology 56 1040 1969
8 DeGroote J Gedigk P Popper H Scheuer P J Thaler H Desmet V J Korb G Poulsen H Schmid M Uehlinger E & Wepler W A classification of chronic hepatitis Lancet 2 626 1968
9 Dietrichson O Nielsen J O Elling P & Christoffersen P The relevance of a serological classification of chronic hepatitis Acta Pathol Microbiol Scand (B) 81 519 1973
10 Doniach D Autoimmunity in liver disease in relation to genes drugs and viruses In Progress in immunology II (ed L Brent and J Holborow) pp 231–243 North Holland Publishing Co Amsterdam 1974
11 Doniach D Roitt I M Walker J G & Sherlock S Tissue antibodies in primary biliary cirrhosis active chronic (lupoid) hepatitis cryptogenic cirrhosis and other liver diseases and their clinical implications Clin Exp Immunol 1 237 1966
12 Dudley F J O Shea M J Ajdukiewicz A & Sherlock S Serum autoantibodies and immunoglobulins in hepatitis associated antigen (HAA) positive and negative liver disease Gut 14 360 1973
13 Farrow L J Holborow E J Johnson G D Lamb S G Stewart J S Taylor P E & Zuckerman A J Autoantibodies and hepatitis associated antigen in acute infective hepatitis Br Med J 3 693 1970
14 Feizi T Immunoglobulins in chronic liver disease Gut 9 193 1968
15 Holborow E J Hemsted E H & Mead S V Smooth muscle autoantibodies in infectious mononucleosis Br Med J 3 323 1973
16 Klatskin G & Kantor F S Mitochondrial antibody in primary biliary cirrhosis and other diseases Ann Intern Med 77 533 1972
17 Lam K C Mistilis S P & Perrott N Positive tissue antibody tests in patients with prolonged extrahepatic biliary obstruction N Engl J Med 286 1400 1972
18 Lidman K Clinical diagnosis in patients with smooth muscle antibodies Acta Med Scand 200 403 1976
19 Mancini G Carbonara A O & Heremans F J Method of single radial immunodiffusion Immunochemistry 2 239 1965
20 Menghini G One second needle biopsy of the liver Gastroenterology 35 190 1958
21 Murray Lyon I M Stern R B & Williams R Controlled trial of prednisone and azathioprine in active chronic hepatitis Lancet 1 735 1973
22 Rubin E Schaffner F & Popper H Primary biliary cirrhosis Am J Pathol 46 387 1965
23 Sherlock S Disease of the liver and biliary system Blackwell Scientific Publications Oxford 1975
24 Soloway R D Summerskill W H J Baggenstoss A H & Schoenfield L J Lupoid hepatitis a nonentity in the spectrum of chronic active liver disease Gastroenterology 63 458 1972
25 Vischer T L Australia antigen and autoantibodies in chronic hepatitis Br Med J 2 695 1970
26 Van Waes L Van Egmond J Van Nummen L Barbier F Wieme R & Demeulenaere L Chronic liver disease and hepatitis B antigen a prospective study Br Med J 3 444 1974

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The Course of Endocrine Ophthalmopathy during Antithyroid Therapy in a Prospective Study

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ABSTRACT In a prospective study the ocular manifestations of 105 thyrotoxic patients were carefully observed and registered during a 24 month antithyroid drug therapy The treatment was supervised very closely and every effort was made to avoid iatrogenic hypothyroidism None of the patients required any ocular surgery and in none did the ophthalmopathy become significantly worse This favourable experience may indicate that a careful antithyroid regimen as outlined is not likely to be accompanied by worsening of the endocrine ophthalmopathy

Key words Eye manifestations hyperthyroidism thyroid antagonists

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The ocular manifestations named endocrine ophthalmopathy (EO) empirically connected with thyrotoxicosis may be found in all types of the disease

The pathogenesis of EO is confusing Immunological mechanisms including a cell mediated immune response have been stressed as a possible basic mechanism (5 6 13) This could explain why orbital involvement may occur in patients who are euthyroid (11) or even hypothyroid (3 4)

In cases of EO class 1 correction of the thyrotoxicosis is generally followed by disappearance of the lid retraction and stare The course of EO classes 2–6 in any patient is totally unpredictable In most patients however the active phase of the ocular disease will last from 3 months to 3 years From this point of view the majority of patients with EO require no heroic measures for a condition that is self limiting and to a variable degree will regress Any treatment of EO seems to be essentially palliative to tide the patient over until spontaneous remission sets in

The purpose of the present investigation has been to ascertain in a prospective study whether gradual attainment of euthyroidism and very careful control of thyroid function with antithyroid drugs appears to be linked with a satisfactory improvement of a co-existent EO In the literature there are no reports of similar prospective studies on the influence of thyroidectomy or radioactive iodine therapy and to our knowledge only one such study of EO during antithyroid regimen has been published (1) The latter study had a very promising outcome and we found it tempting to check the results in a similar prospective investigation

PATIENTS AND METHODS

This investigation concerns a series of 105 thyrotoxic patients admitted to the Akershus Central Hospital 1968–77 The female/male ratio was 84/21 and the average age was 41 years (range 14–72)

A complete medical evaluation was made before the patients were included in the study A series of tests of thyroid function was recorded in each patient including the 2 and 24-hour uptake of ^{131}I serum protein bound and butanol-extractable iodine and the measurement of Achilles reflex ½ relaxation time with photomotogram In recent years these tests were supplemented or replaced by total serum triiodothyronine total serum thyroxine and thyrotropin releasing hormone tests Blood samples of all patients were examined for thyroid antibodies (thyroglobulin and microsomal antibodies)

Each patient was interviewed and examined before and at the end of the antithyroid drug therapy by the same ophthalmologist (J Y) who also re-examined the patient in the event of debatable changes in the ophthalmic picture

Independently of the examination in the Department of Ophthalmology Ulleval Hospital Oslo symptoms and signs of EO when present were registered with great c

Table I *Size of goitre*

EO=endocrine ophthalmopathy

	No of pats	
	Without EO	With EO
No goitre	10	16
Minimal goitre (palpable but not visible 35 g or less)	12	14
Moderate goitre (up to 60 g)	8	30
Large goitre (60 g or more)	6	9
Total	36	69

by the internist from the very first visit The eye changes were recorded according to Werner s modification of the classification adopted by the American Thyroid Association (14) The abridged classification was as follows

0=No signs no symptoms 1=No symptoms only signs as stare lid lag or lid retraction 2=Soft tissue involvement with symptoms and retrobulbar discomfort sandy sensation and increased lacrimation signs of puffiness filling of the upper orbito-palpebral sulcus (positive sulcus sign) conjunctival injection oedema of conjunctivae (chemosis) or visible increase of lacrimal glands 3=Exophthalmos (proptosis as *defined by Werner (15)*) 4=Extraocular muscle involvement usually diplopia (ophthalmoplegia) 5=Corneal involvement 6=Sight loss (optic nerve involvement)

As soon as a patient entered the study the pretreatment classification was confirmed by filling in a standardized form At each visit specific inquiries were made about *details of the EO* as outlined by Werner (14) The chart was brought up to date consecutively with regard to possible symptoms and signs related to classes 1–4 Classes 5 and 6 with corneal involvement and sight loss respectively were omitted because no patient in the series belonged to either of them

Thyroid size was evaluated by clinical assessment and thyroid scanning The size of goitre is given in Table I and the degree of thyrotoxicity in Table II

All patients were treated with antithyroid drugs for 24 months The initial drug was carbimazole in a dosage of 10

Table II *Degree of thyrotoxicity at start of treatment*

EO=endocrine ophthalmopathy

	No of pats	
	Without EO	With EO class 1–4
Mild	23	22
Moderate	9	28
Severe	4	19
Total	36	69

Table III *Ocular symptoms and signs in 69 patients with endocrine ophthalmopathy (pretreatment observations)*

Symptoms	
None	17
Retrobulbar discomfort	14
Sandy sensation	25
Increased lacrimation	22
Blurring	12
Diplopia	7
Signs	
None	0
Stare	44
Lid lag	26
Upper lid retraction	8
Puffiness	11
Sulcus sign positive	37
Conjunctival injection	15
Chemosis	8
Increase of lacrimal glands	8
Exophthalmos	26
Extraocular muscle involvement	12

mg 3 times or 5 mg 4–6 times a day Irrespective of the activity of the ophthalmic process this dosage was administered until a euthyroid state approached whereupon the dosage was gradually reduced in order to attain a steady state of euthyroidism In 8 patients carbimazole was replaced by propylthiouracil in equivalent doses during the early phase of the treatment due to drug allergy (rash) Every effort was made to avoid hypothyroidism during treatment and in no case were thyroid hormones added to the antithyroid medication

The pretreatment ocular symptoms and signs of the 69 patients with ophthalmopathy 17 of whom had involvements of class 1 only are listed in Table III Table IV records the distribution of the initial measurements of proptosis All exophthalmometer readings were made by the same observer (J Y) with the same instrument (9) a Hertel exophthalmometer To judge from the pretreatment observations 36 patients had no ophthalmopathy

RESULTS

Thyrotoxicosis was effectively controlled in all 105 patients by appropriate administration of antithyroid drugs

None of the 69 patients with EO in this series of 105 patients required decompression of the orbit because of the ophthalmic process None developed impairment of vision during treatment No patient who did not have ophthalmoplegia prior to therapy suffered from it after treatment

Chemosis and conjunctival injection usually diminished gradually when the EO stabilized Filling or obliteration of the sulcus however persisted

Table IV *Initial proptosis (mm) measured on more prominent eye with Hertel exophthalmometer*

Ophthalmopathy	12–14	15–19	20–24	25–29
Class 1 (n 17)	1	9	7	
Classes 2–4 (n=52)	5	26	19	2

Table V *Maximal difference between initial and final measurement of proptosis*

Ophthalmopathy	Decrease 3 mm or more	Insignificant increase or decrease (2 mm or less)	Increase 3 mm or more
Class 1 (n=17)	1	16	
Classes 2–4 (n 52)	3	44	5

hroughout the study in about one third of the patients with infiltrative EO

The differences in proptosis before and after treatment are shown in Table V In most patients the maximal increases were very moderate and in a few cases increases in proptosis were later followed by a decrease The latter phenomenon was observed in three of the 5 patients who showed the greatest increase in proptosis (3–4 mm) In all 5 patients the symptoms were relieved by raising the head of the bed at night and by simple use of eye drops

Concerning the definition of exophthalmos (14 15) exophthalmometry with Hertel s instrument is a rather crude method The significance of the sulcus sign as an early manifestation of infiltrative EO has been emphasized and is said to be of more importance than the initial exophthalmometer values (9) The present study confirms this observation In most cases the patient s complaints were more related to the initial clinical eye signs such as puffiness and filling of the sulcus than to the first exophthalmometer readings

Of the 17 patients with EO class 1 before treatment none had changed to classes 2–4 at the end of therapy In many of them however stare lid retraction and/or lid lag persisted for years after the eumetabolic state had been established through antithyroid drug treatment None of the 36 patients judged to be free from ophthalmopathy before treatment suffered from it after therapy

Analysis of the patients with EO showed no correlation between the antibody titres of either thyroglobulin antibodies or of microsomal antibodies and the severity of the ocular signs

DISCUSSION

The clinical feature and course of EO are variable and unpredictable The assertion of Brain (2) "There is an exception to every statement that can be made about exophthalmic ophthalmoplegia still holds good 25 years later

Severe progressive eye changes may accompany euthyroid and even hypothyroid individuals (3) The overall impression however is that the majority of patients with EO have clinical evidence of thyrotoxicosis and control of associated hyperthyroidism is commonly accepted as the basic part of the treatment of the eye changes

It has been stressed that hypothyroidism should not be allowed to develop during treatment for EO because hypothyroidism aggravates the ocular disorder (7 8) A slow medical thyroidectomy achieved by antithyroid drugs has therefore been advocated (9) since it provides a flexible and reversible form of treatment and allows therapy to be titrated against thyroid status

Most of the published therapeutic trials with antithyroid drugs have been retrospective and the results are difficult to interpret The most thorough study of the ocular manifestations of thyrotoxicosis is that of Aranow and Day (1) They made detailed observations and careful measurements at frequent intervals on 129 patients with mild and severe forms of EO treated with an antithyroid drug only These authors attributed their favourable experience to their deliberate attempt to control the thyroid status in a careful way

The results in our series were encouraging and may suggest that the antithyroid drug program as outlined is less likely to be followed by a worsening of the ophthalmopathy of thyrotoxic patients than either thyroidectomy or radioactive iodine therapy Our results thus confirm the findings and conclusions of Aranow and Day

Parry s first description (10) of a patient with the condition we now call Graves or Basedow s disease included a psychic trigger to the illness Although it has been difficult to prove this relation most

endocrinologists seeing large numbers of patients with the disease are convinced that such a cause-and-effect relation often exists Volpe who recently reviewed this subject (12) refers to the evidence that thyroxine itself may affect T lymphocytic function Emotion might in this way act in the syndrome including the EO through an influence on immune mechanisms

It does not seem probable that our favourable experience was purely fortuitous It is however impossible to judge definitely what part the antithyroid drug program as outlined above played in the outcome Our findings might equally well be used as evidence that the reassurance provided by frequent attention and sympathetic interest may have played a decisive role

REFERENCES

1 Aranow H & Day R M Management of thyrotoxicosis in patients with ophthalmopathy Antithyroid regimen determined primarily by ocular manifestations J Clin Endocrinol 25 1 1965
2 Brain R Discussion on the management of endocrine exophthalmos Proc R Soc Med 45 237 1951
3 — Pathogenesis and treatment of endocrine exophthalmos Lancet 1 109 1959
4 Brownlie B E W Newton O A G & Sing S P Ophthalmopathy associated with primary hypothyroidism Acta Endocrinol (Kbh) 79 691 1975
5 Doniach D The pathogenesis of endocrine exophthalmos a short review Proc R Soc Med 70 695 1977
6 Doniach D & Florin-Christensen A Autoimmunity in the pathogenesis of endocrine exophthalmos Clin Endocrinol Metabol 4 341 1975
7 Havard C W H Endocrine exophthalmos Br J Med I 360 1972
8 Ingbar S H Large doses of radioiodine in the treatment of thyrotoxicosis N Engl J Med 279 1395 1968
9 Lamberg B A The thyro-hypophysial syndrome I The primary reaction of the hypophysial eye signs (including exophthalmos) to the treatment of thyrotoxicosis Acta Med Scand 148 225 1954
10 Parry C H Enlargement of the thyroid gland in connection with enlargement and palpitation of the heart Collections from the Unpublished Medical Writings vol 2 p 11 Underwoods London 1825
11 Solomon D H Chopra I J Chopra U & Smith F I Identification of subgroups of euthyroid Graves s ophthalmopathy N Engl J Med 296 181 1977
12 Volpe R The role of autoimmunity in hypoendocrine and hyperendocrine function with special emphasis on autoimmune thyroid disease Ann Intern Med 87 86 1977
13 Volpe R Edmonds M Lamki L Clarke P V & Row V V The pathogenesis of Graves disease A disorder of delayed hypersensitivity Mayo Clin Proc 47 824 1972
14 Werner S C Classification of the eye changes of Graves disease J Clin Endocrinol Metab 29 982 1969
15 — Modification of the classification of the eye changes of Graves disease Recommendations of the Ad Hoc Committee of the American Thyroid Association J Clin Endocrinol Metab 44 203 1977

Iodine-Induced Hypothyroidism and Its Effect on the Severity of Asthma

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ABSTRACT Among 1 107 patients with asthma or chronic asthmatic bronchitis and 96 with hypothyroidism we found 12 patients with both diseases Seven of these patients had hypothyroidism induced by iodine containing expectorants Five of 209 patients with hyperthyroidism had coexistent asthmatic lung disease but none of these five had been taking iodine The severity of asthma is considered in relation to changes in thyroid function following withdrawal of iodine Three patients had an exacerbation of their asthma, while another three had no change in their asthma when the iodine treatment was discontinued and the euthyroid state subsequently restored All six patients had a marked improvement of their general wellbeing when the euthyroid state was re established It is concluded that treatment with iodine-containing expectorants causing hypothyroidism is unwise and should be withdrawn even when the patients praise the beneficial effect of iodine on the asthma per se

Key words hypothyroidism iodine asthma

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Coexistence of thyroid disease and asthma is of course occasionally seen and several reports have established a relationship between the thyroid state and the severity of the asthma Hyperthyroidism is usually associated with a worsening of the asthma with a subsequent improvement during treatment of the hyperthyroidism Development of hypothyroidism usually improves the coexistent asthma which again returns to the usual state during thyroid replacement treatment

Development of reversible hyper or hypothyroidism during long term treatment with iodine containing drugs such as iodine containing expectorants has been reported several times Recently we had the opportunity of seeing an asthmatic patient with severe hypothyroidism who had a pronounced and persistent worsening of her asthma when the iodine expectorant was discontinued and the thyroid function subsequently restored

Apparently amelioration and exacerbation of asthma registered by patients treated with iodine expectorants has not been considered before in relation to changes in the thyroid state induced by iodine This study deals with the problem and the mechanism is found to be a plausible explanation in some cases

PATIENTS AND METHODS

In a retrospective study asthma or chronic asthmatic bronchitis was recorded in 1 107 patients admitted to our Medical Department during a 4½ year period Hypothyroidism was recorded in 96 patients Coexistence of the two diseases was found in 12 patients eight women and four men aged 46–81 years (average 62) Hyperthyroidism was recorded in 209 patients and five of these had coexistent asthma or chronic asthmatic bronchitis

The thyroid function was assessed on admission when the patient s iodine intake was stopped and again ¾–11 months later Thyroxine (T_4) triiodothyronine (T_3) and thyroid stimulating hormone (TSH) were determined by radioimmunoassay on unextracted serum

The state of asthma was assessed by careful questioning and repeated physical examinations in hospital and later in our Our Patient Department

RESULTS

Seven of the 12 patients who had hypothyroidism coexistent with asthma or chronic asthmatic bronchitis had been taking iodine expectorants almost constantly during a period ranging from three to more than ten years The daily dose of iodine varied from 300 to 1 000 mg After discontinuation of iodine in six of the patients the thyroid

Abbreviations T = thyroxine T_3 = triiodothyronine TSH = thyroid stimulating hormone

Table I *Thyroid parameters and change in the severity of asthma in seven patients with reversible hypothyroidism induced by iodine containing expectorants*

Case no	Sex	Age (y)	Iodine dose (mg/d)	Duration of therapy (y)	Thyroid tests during iodine intake T_4 (nmol/l)	T_3 (nmol/l)	T_3 test	TSH (mU/l)	Thyroid tests and state of asthma after iodine intake Mo after	T_4 (nmol/l)	T_3 (nmol/l)
1	♀	46	600	4	10	0 50	0 65	52	½	80	1 95
2	♀	51	650	4	10	1 40	0 75	32	4	102	2 20
3	♂	70	800	12	56	0 60	1 17	?	1	108	1 35
4	♀	48	800	5	49	1 00	0 71	19	1½	117	1 70
5	♂	73	1 000	3	7	?	0 87	52	1½	56	2 15
6	♀	69	500	10	53	1 20	?	11	11	84	1 75
7	♂	79	300	15	52	0 95	0 95	20	*	*	*
Reference values					66–139	1 14–2 30	0 90–1 10	0–3 0			

* Parameters on replacement treatment

function assessed clinically and by biochemical parameters was restored within a period varying from three weeks to 11 months (Table I) One patient (no 7) absolutely refused to cooperate and continued the iodine ingestion together with thyroid replacement therapy In one patient (no 1) with severe hypothyroidism there was a pronounced and persistent worsening of the asthma when the iodine was discontinued and the thyroid function restored Permanent treatment with prednisone was found necessary in this patient who had never before required treatment with steroids In o patients (nos 2 and 3) the discontinuance of odine and the subsequent restoration of the euthyroid state was followed by a moderate worsening of the asthma but permanent treatment with additional bronchodilators had a satisfactory effect Another three patients (nos 4 5 and 6) experienced no change in the asthma when the euthyroid state was re established

Coexistence of hyperthyroidism and asthma or chronic asthmatic bronchitis was found in five patients only none of whom had been treated with iodine recently

DISCUSSION

Daily intake of iodine has for a long time been known to be one of many important factors influencing thyroid function but the effect of iodine on the thyroid hormone synthesis and release is very complex and rather unpredictable even in patients free from previous disorders of the thyroid gland Reports of thyrotoxicosis (10) as well as hypothyroidism (1 2, 12) induced by chronic iodine intake are numerous but in connection with a high daily intake of iodine containing expectorants in asthmatic patients hypothyroidism is by far the most common complication This observation is confirmed by our finding of seven patients with hypothyroidism and no patients with hyperthyroidism induced by a daily intake of iodine in doses of 300–1 000 mg A latency of several years before the development of hypothyroidism is in accordance with what was found by the authors mentioned above The long latency is in contrast to that often seen in the development of thyrotoxicosis during iodine intake (10 13) A subsequent restoration of the euthyroid state after discontinuing the high iodine intake was seen in most earlier reports (5 9 12) and all our patients had a normal thyroid hormone level after a period ranging from three weeks to 11 months (Table I) Whether our patients developed hypothyroidism because of an underlying subclinical autoimmune thyroiditis as suggested by Hall et al (4) we do not know but three of the patients did have thyroid autoantibodies in their blood Clinically however the patients were considered free from previous disorders of the thyroid gland

Asthma has recently been reported to be relieved by hypothyroidism (3) and conversely thyrotoxicosis has for several years been known to cause exacerbation of coexistent asthma (11) The biochemical mechanisms responsible for this relationship are unclear but the thyroid hormone is

T_3 test	TSH (mU/l)	Worsening of asthma
0 89	45	Pronounced
0 94	0 4	Moderate
1 03	2 2	Moderate
0 82	4 0	No
0 98	20	No
?	5 4	No
?	?	?

known to influence tissue responsiveness to catecholamines (8) tissue and plasma concentrations of cyclic adenosine 3 5 monophosphate (7) and metabolism of hydrocortisone (6) factors which are of great importance for the severity of asthma We have not been able to find evidence that iodine has any direct effect on the biochemical processes mentioned

Amelioration of asthma in patients taking iodine containing expectorants has apparently never been considered before in relation to the subsequent suppression of the thyroid gland and conversely the exacerbation of the asthma after stopping iodine intake has not before been associated with the restoration of a normal thyroid function The clinical course in three of our patients strongly suggests that such a mechanism must be taken into consideration Of course not all patients receiving long term treatment with iodine expectorants develop hypothyroidism but according to the investigations by Begg and Hall (2) a considerable proportion do A resumption of treatment with iodine expectorants certainly cannot be recommended to patients who have formerly developed hypothyroidism as changes in the thyroid function may take place very rapidly Hypothyroidism may return within a few weeks after resuming the ingestion of iodine (9)

Although three of our patients had a worsening of their asthma in connection with the restoration of the euthyroid state it must be underlined that all six patients had a marked improvement of their well being In particular they were very happy to be rid of the weakness and fatigue which most of the patients thought were caused by the lung disease Certainly none of the patients wanted to return to the hypothyroid state

The conclusion of course is that treatment with iodine expectorants causing hypothyroidism is unwise Even when patients praise the beneficial effect of iodine expectorants on the asthma per se one should not approve such a treatment

REFERENCES

1 Asklund M & Østergaard Kristensen H P Jodmyksødem Ugeskr Laeger 128 1555 1966
2 Begg T B & Hall R Iodine goitre and hypothyroidism Q J Med 32 351 1963
3 Bush R K Erlich E N & Reed C E Thyroid disease and asthma J Allergy Clin Immunol 59 398 1977
4 Hall R Turner Warwick M & Doniach D Autoantibodies in iodine goitre and asthma Clin Exp Immunol 1 285 1966
5 Helgason Th Iodine goitre and myxoedema in chronic respiratory disorders Br J Dis Chest 58 73 1964
6 Hellman L Bradlow H L Zumoff B & Gallagher T F The influence of thyroid hormone on hydrocortisone production and metabolism J Clin Endocrinol Metab 21 1231 1961
7 Karlberg B E Henriksson K G & Andersson R G G Cyclic adenosine 3 5 monophosphate concentration in plasma adipose tissue and skeletal muscle in normal subjects and in patients with hyper and hypothyroidism J Clin Endocrinol Metab 39 96 1974
8 Murray J F & Kelly J J The relationship of thyroidal hormone level to epinephrine response A diagnostic test for hyperthyroidism Ann Intern Med 51 309 1959
9 Nixon P G F Recurrent myxoedema and goitre attributed to potassium iodine Br Med J 1 748 1957
10 Savoie J C Massin J P Thomopoulos P & Leger F Iodine induced thyrotoxicosis in apparently normal thyroid glands J Clin Endocrinol Metab 41 685 1975
11 Settipane G A Schoenfield E & Hamolsky M H Asthma and hyperthyroidism J Allergy Clin Immunol 49 348 1972
12 Taguchi J T & Skillman T G Iodine induced myxoedema Report of a case and review of the literature Am J Med Sci 239 417 1960
13 Thorsteinsson B & Kirkegaard C Iodine induced hyperthyroidism and bronchial asthma Lancet 2 294 1977

BOOK REVIEW

Pheochromocytoma By W M Manger and R W Gifford 168 figures 56 tables approx 350 pages Cloth DM 113 10 US $49 80 Springer Verlag Berlin Heidelberg and New York 1977 ISBN 3 540-90217 1

Among the many recent monographs on interesting and important diseases this recent volume deserves a special place for many reasons The subject has great theoretical importance but its practical implications are unique as it offers a chance to cure malignant hypertension definitely The study of this disease has early taught us the important lesson that severe vascular changes in the ocular fundi are reversible when the hypertensive agent is removed by successful operation This has been an important fact for the understanding of hypertensive disease Pheochromocytoma was the second tumor that could be diagnosed from examination of specific biochemical products excreted in the urine

The volume contains a dedication to different workers in the field First among them is Ulf von Euler In Scandinavia we should remember that the clinician Arthur Engel in Falun and the physiologist Ulf von Euler in Stockholm together made the first diagnosis on a patient with this disease after determination of catecholamines in the urine The operation was successful

This volume contains a wealth of information not only regarding neoplasia from the chromaffin tissue in the adrenal marrow but also regarding a great number of other diseases that are in some way connected with the main subjects I have a strong feeling that practically nothing has been omitted Especially interesting is chapter 7 with a detailed analysis of a great number of conse utive patients illustrating different aspects of the disease This chapter gives a vivid picture of the variable clinical manifestations also when compared to the absolute amount of catecholamines in tumor and plasma The next chapter contains very valuable information regarding the operative treatment It is beautifully illustrated and on the whole it may be said that the layout of the book is superb with an abundance of color plates and graphs illustrating important problems and clinical and morphological findings

It is clear that all details cannot be of the same high quality Personally I would say that the chapter on carcinoid tumors contains a number of statements that I would not accept but on the whole the discussion of differential diagnosis seems to be very good

Compared to the general quality of the volume that is really non plus ultra the price is not too high I am convinced that the book will be consulted most profitably not only by professional endocrinologists but also by all doctors and basic scientists who are interested in the fundamental problems of hypertension

Jan G Waldenstrom

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LETTERS TO THE EDITOR

Sir

I would like to comment on three points raised in the thoughtful and challenging paper by Friis and colleagues that appeared recently (1)

Firstly the authors suggest that the single target strategy (STS) for diagnosis is preferable to other strategies However STS is only sensible if the doctor is very certain about the diagnosis and if the time in making an alternative diagnosis should the initial one be incorrect is not important In patients with haemoptysis it is usually advisable to have a bronchoscopy even though a pneumonia is the most likely cause The consequences of missing a bronchial cancer are serious enough to make it worthwhile doing this investigation

Consider the following idealised situation A clinician judges a patient to have a 70% chance of a gastric ulcer (GU) or a 30% chance of a gastric carcinoma Assume a gastric carcinoma is 10 times worse than a GU (a very modest assumption) On probability alone the clinician would go for a GU but considering both factors he would look for the carcinoma (3 units of diagnostic desirability v 0 7 units)

The degree of certainty about a diagnosis depends on the experience of the clinician Whilst it may be most efficient for all patients to be seen by a senior member of staff this practice does not provide a learning opportunity for the junior staff Where are the future senior members of staff to come from?

The degree of certainty about a diagnosis depends on the a priori probability of the diagnosis occurring If the doctor is sent patients with a relatively small range of conditions such as in a specialised clinic then his diagnostic accuracy would be higher than someone who sees patients with a wide range of conditions even though the experience of the two doctors may be equivalent in their relative fields STS is best employed for conditions which have a well recognised clinical presentation such as angina peptic ulcers prostatism which are relatively common in the population and where presentation is unlikely to be mimicked by potentially fatal disease

An alternative and better approach is the single diagnostic strategy (SDS) in which a particular investigation rather than a particular disease is considered The diagnostic yield would be very high if endoscopy has been carried out in all patients with epigastric pain Similarly if CAT scans have been employed in all patients with cerebral disease the diagnostic accuracy would be very high This strategy would solve the dilemma of balancing frequency against severity posed in the idealised situation given above

Secondly the question of the hospital record containing more information than is needed is based essentially on a retrospective judgement of what data did actually contribute to the diagnosis This judgement can only be made when the diagnosis is known If it is possible to predict exactly what information is needed in the individual patient then the diagnosis could be predicted in that patient For the two are equivalent

The traditional manner in which medical students are taught to take a full history and clinical examination is still relevant and highly important The clinical examination has two purposes Firstly to screen for possible disorders by enquiring about a range of symptoms and secondly to seek specific information about those conditions which seem likely in the patient This twofold process inevitably means that a lot of irrelevant information is collected Unless embryo doctors learn the first part of the process they will never perform the second adequately

Collecting a lot of information does not necessarily mean that one is using an inductive process to reach the likely diagnosis Hypotheses can be based on various items of information in the record while other items are rejected as irrelevant The problems orientated system can be regarded as making a set of hypotheses from a clinical record Though it is said quite clearly that the problem list should be a list of certainties quite commonly it contains such items as congestive cardiac failure asthma duodenal ulcer which are arrived at not by specific investigations but by hypothesis from the clinical picture

It could be argued that STS is more likely to demand an inductive approach than a deductive one As the clinician is encouraged to make a diagnosis from the initial data and not to perform a range of investigations to seek further information

Yours sincerely

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REFERENCE

1 Friis H Hansen S W Binder V Riis P & Wulff H R An arm chair study of diagnostic decision making in gastroenterological out patients Acta Med Scand 203 149 1978

Sir

First of all we would like to point out that our paper had a limited scope We only set out to study what would happen if a skilled physician directed his diagnostic efforts towards the most likely diagnosis and not towards all possible diagnoses The study was made on non acute patients in the out patient department where the time factor is less important if only the necessary investigations are carried out within a limited number of days In acute in patients the time factor is often essential and it may well be necessary to adopt a different strategy It may for instance be advisable to do a lumbar puncture in a febrile patient even when meningitis is considered an unlikely possibility

Dr Young adopts a decision theory approach to the diagnosis of gastric cancer but we feel that this particular example has little do to with diagnostic strategy Patients suspected of having a benign ulcer as well as patients suspected of malignant ulceration must be subjected to gastroscopy in order to establish the diagnosis with certainty Thus the investigation to be made is the same regardless of our prior probability of the nature of the ulcer We object however to the idea that a patient with for instance epigastric pain is *at the same time* subjected to a great number of different investigations each aiming at different diagnostic possibilities

We agree with Dr Young as regards his concern for the education of medical students and doctors and of course we did not suggest that new patients are *only* seen by a senior member of the staff We suggest that the patient is seen *also* by a senior member who then discusses the case with his younger colleague We also think that we might make a greater effort to teach medical students and junior colleagues how to extract the relevant information from all the routine data and to analyse their reasons for the diagnostic tactics which they suggest in the individual case

Dr Young suggests what he calls a single diagnostic strategy but we feel that both the examples which he mentions must be subjected to a critical cost–benefit analysis and we suspect that they would not stand this test

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Poisoning with Brown Fly Agaric, Amanita Regalis

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ABSTRACT Three patients ate different amounts of a common northern mushroom, brown fly agaric, Amanita regalis All of them believed they had eaten delicious parasol mushrooms, Macrolepiota procera The symptoms of poisoning began 1-2 hours after ingestion of the mushrooms All the patients had marked gastrointestinal symptoms nausea and heavy vomiting Two had central nervous system manifestations and cholinergic symptoms hallucinations confusion, or loss of consciousness as well as copious salivation, or sweating All patients recovered within 4-24 hours without any damage to liver kidneys or central nervous system It seems that cooking the mushrooms does not completely neutralize the toxic agents of Amanita regalis The analysis of fried mushrooms shows that it may be possible to identify mushrooms reliably from the remains of a meal

Poisoning due to brown fly agaric (Amanita regalis (Fr) Maire) is unusual This mushroom species is sometimes treated as a northern variant of red fly agaric (Amanita muscaria (Fr) Hook) with the name Amanita muscaria var umbrina (Fr) (3) The species is common in Finland (7) Amanita regalis may induce gastrointestinal symptoms but it is not known whether like Amanita muscaria it has any effect on the central nervous system (2) The muscarine content of Amanita muscaria has been considered to be too small to produce any marked intoxication symptoms (1) The toxins of Amanita regalis have not been analyzed chemically

We describe three patients with poisoning due to Amanita regalis In addition to gastrointestinal symptoms two of them had a marked central nervous system affection and a strong cholinergic syndrome All the patients believed they had eaten an edible species parasol mushroom (Macrolepiota procera (Fr) Sing)

CASE REPORTS

Case 1

A 27 year-old previously healthy woman ate several fried mushrooms believing them to be Macrolepiota procera About two hours later she experienced nausea and vertigo and was admitted to the emergency room at Helsinki University Central Hospital Soon after admission she lost consciousness but responded to painful stimuli with purposeful movements and muscle twitchings She was obviously hallucinating Pupils were normal in size and responded normally to light The ocular fundi were normal The tendon reflexes were brisk symmetric the Babinski sign negative She vomited heavily several times Salivation was copious during the first hours in hospital Some buccomasticatory movements were noted BP was 140/80 heart rate 85/min The rectal temperature was low 35 5 C

Routine laboratory analyses including creatinine transaminases alkaline phosphatase sodium potassium chloride and urine analysis were normal Astrup analysis showed a transient mild metabolic acidosis with pH 7 30 pCO_2 43 standard bicarbonate 20 and base excess −5 1 Liquor was normal in its cell protein and glucose contents ECG was normal

A gastric lavage showed an abundant amount of pieces of mushrooms The patient was further treated with 50 g of activated charcoal as well as with i v infusions with 0 9% saline and 5% glucose

The unconscious state lasted for 12 hours Nausea and vertigo continued for 12 more hours Later she felt completely well Physical examination after recovery was normal At the check up one week later no evidence of central nervous system liver or kidney damage was seen at the physical examination or in the laboratory analyses

Remains of the mushroom meal had been frozen at −20 C and were analyzed later in the Department of Botany University of Helsinki A great many of the pieces had a typical ring in their stem and could be shown to belong to the genus Amanita The cap had a yellow brown surface with large light yellowish scales which indicate Amanita regalis Microscopic examination revealed oval light smooth and apiculate spores Melzer s solution showed them to be inamyloid

The anamnestic data given by the patient and her friend after recovery as well as the examination of the remains of the meal confirmed that the patient had eaten several large specimens of Amanita regalis

Case 2

A 55-year-old man with a previous inferior myocardial infarction slight renal insufficiency and glaucoma ate about two cooked mushrooms He felt nausea after two hours and vomited heavily four hours after the meal On admission to Helsinki University Central Hospital four hours after the meal he was at times disoriented and hallucinating. He sweated profusely BP was 120/60 heart rate 65/min. Pupils were small due to the use of pilocarpine The ocular fundi were normal The hallucinations and confusion continued for the next 12 hours but consciousness was maintained The rectal temperature was low 35 1°C

The mushrooms ingested were approximately 10 to 15 cm tall The cap of the younger mushroom was convex that of the older one more flat light brown with brown spots The stem was supplied with a ring. The macroscopic appearance of the mushrooms eaten corresponds to Amanita regalis

The patient was treated with 50 g of activated charcoal and fluid infusions After recovery no evidence of central nervous system liver or further kidney damage was observed at the physical examination or in the repeated laboratory analyses The patient was discharged 6 days after ingestion of the mushrooms

Case 3

A 53-year-old previously healthy woman ate a small amount of cooked mushrooms The anamnestic macroscopic appearance of the mushrooms fits Amanita regalis Within one hour she vomited heavily

At physical examination three hours later she was quite well after several vomits She was treated at Helsinki University Central Hospital for 20 hours Activated charcoal was given in a dose of 50 g No evidence of central nervous system liver or kidney damage developed the physical status or in the laboratory parameters

DISCUSSION

The present results show that the mushroom Amanita regalis has marked effects on the central nervous system with confusion hallucinations and loss of consciousness The cholinergic syndrome includes vomiting salivation and sweating The results also show that it is possible though not easy to indentify mushrooms reliably from the remains of a meal

All three patients believed they had eaten an edible species of mushroom Macrolepiota procera This species has been considered so easy to identify that it features in a list from 1971 of 30 commercial species recommended by the Finnish Committee for the utilization of mushrooms Laymen often fail to analyze the details of mushrooms precisely which makes errors like these possible

The amount of muscarine in Amanita muscaria has been estimated to be 0 0002% of the fresh mushroom which is too low for marked toxic effects (1) It is known however that many factors in the environment as well as the age of the mushroom influence its toxicity This may explain the strong cholinergic syndrome in our patients It is also possible that some species of the genus Amanita may contain other muscarine like substances too (1) Because the toxins of Amanita regalis have not yet been analyzed the chemical background of these symptoms remains an open question

The effects of Amanita muscaria on the central nervous system have been attributed to ibotenic acid and its decarboxylated derivative muscimol (2) These agents are devoid of cholinergic properties In experimental animals they induce excitation mydriasis cramps and hyperpnea followed by sedation and sleep In addition they have some antiemetic and antitussive effects (1) EEG changes have been shown in rabbits (5) In human experiments muscimol given in peroral doses of 7 5–10 mg has produced ataxia muscle twitchings euphoria dysphoria and a psychotic state with depersonalization and derealization (6) Thus it is possible that ibotenic acid and muscimol are the principal toxins of Amanita regalis too affecting the central nervous system

The relatively mild symptoms in patients 2 and 3 are probably explained by the mushrooms having been cooked This procedure believed to destroy the toxic agents of Amanita muscaria apparently does not completely neutralize those of Amanita regalis

The poisoning due to Amanita regalis seems to be relatively mild and of short duration A complete recovery is to be expected No evidence of permanent organ damage was found in our patients Marked possibilities of complications exist however e g copious salivation and vomiting in an unconscious or confused patient with the risk of aspiration and disturbances in the water and electrolyte balances

REFERENCES

1 Eugster C Chemie der Wirkstoffe aus dem Fliegenpilz (Amanita muscaria) Fortschr Chem Org Naturst 27 261 1969
2 Gulden G & Schumacher T Giftsopper og soppforgiftninger Universitetsforlaget Oslo 1977
3 Moser M Die Rohrlinge und Blatterpilze (Agaricales) In Kleine Kryptogamenflora II b/2 3rd ed (ed H Gams) p 443 Fischer Stuttgart 1967

4 Schwietzer C Pilzgifte und Pilzvergiftungen Munch Med Wochenschr 112 1085 1970
5 Scotti de Carolis A Lipparini F & Longo V Neuropharmacological investigations on muscimol a psychotropic drug extracted from Amanita muscaria Psychopharmacol 15 186 1969
6 Theobald W Buch O Kunz H Krupp P Strenger E & Heimann H Pharmakologische und experimental psychologische Untersuchungen mit 2 Inhaltsstoffen des Fliegenpilzes (Amanita muscaria) Arzneim Forsch 18 311 1968
7 Ulvinen T Suursieniopas Suomen Sieniseura Helsinki 1976

Association of Hyperthyroidism with Idiopathic Thrombocytopenic Purpura and Haemolytic Anaemia

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ABSTRACT Two cases of thyrotoxicosis associated with acute idiopathic thrombocytopenic purpura and haemolytic anaemia are reported The underlying cause of the hyperthyroidism was lymphoid thyroiditis and the severe thrombocytopenia was due to an extremely short platelet survival as determined with the ^{51}Cr labelling technique The amount of megakaryocytes was increased in bone marrow sections indicating increased platelet production Treatment with high doses of corticosteroids did not increase the platelet count, which remained at critical levels below 10×10^9/l, and there were still signs of increasd bleeding tendency Splenectomy was therefore considered at an early stage after diagnosis and was performed when the thyrotoxicosis had been brought under control with corticosteroids and β blocking agents A method which has proven useful in minimizing bleeding during surgery in patients with low platelet counts is described This method takes advantage both of the fact that normally 30 % (in cases with splenomegaly even more) of the total body platelets are harboured in the spleen and can be expelled with i v epinephrine, and of the good haemostatic effect of small amounts of platelet concentrates given at appropriate times during the operation It is proposed that splenectomy should be performed early if large doses of corticosteroids do not raise the platelet count above critical levels or if the bleeding continues Partial control of the thyrotoxic state should be obtained with β blocking agents and corticosteroids and measures should be undertaken to minimize the bleeding risks during surgery Underlying immunologic mechanisms responsible for the development of this disease combination are discussed It is suggested that despite the great array of antibodies present, the basic abnormality is confined to immunocompetent T lymphocytes

Key words Hyperthyroidism thrombocytopenia thrombokinetics

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It was Harrington s classic experiments in 1951 which first yielded strong evidence that the plasma in 60% of patients with idiopathic thrombocytopenic purpura (ITP) contained a factor capable of producing thrombocytopenia when transferred to normal recipients (16) Ancillary evidence was obtained from the history of mothers with ITP who frequently give birth to transiently thrombocytopenic children (12 28) Recent methods for in vitro detection of antiplatelet antibody in immune platelet disorders have demonstrated the presence of antiplatelet antibodies in 65% of patients with ITP (19) It is also well documented that platelet survival is characteristically shortened in ITP (2 5 25) and it is assumed that platelet antibodies cause the reduction of platelet survival It was also noted that patients with Coombs positive (autoimmune) haemolytic anaemia sometimes had episodes of ITP suggesting a common etiology (13) Since 1931 sporadic reports have appeared noting an apparent association between hyperthyroidism and ITP (14 18 21 23) and it has been suggested that these two disorders may share a similar immunologic background (23)

ITP with low platelet counts and fully developed hyperthyroidism are both conditions which may endanger the life of the patient Therapeutic measures against the hyperthyroidism might however aggravate the ITP and vice versa Patients with this disease combination must be treated with caution and in a proper sequence although without delay to get the acute situation under control

The present study reports the clinical and laboratory findings in two patients with hyperthyroidism haemolytic anaemia and severe ITP

Abbreviations ITP=idiopathic thrombocytopenic purpura MLS=mean life span TSH=thyrotropin

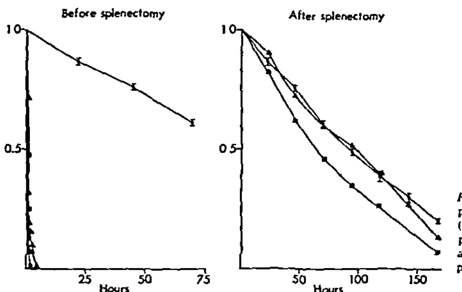


Fig 1 Survival of ^{51}Cr labelled platelets in 16 control subjects (●–● mean ± S E) and in the present two patients before and after splenectomy (▲—▲ = pat 1 ■—■ = pat 2)

not responding to corticosteroid treatment A practical form of therapy is described and the immunologic background common to the three dis orders is discussed

CASE REPORTS

Case I

A 48-year-old married woman was admitted to the hospital se of severe anaemia and hyperthyroidism She had well until six months before admission when she noticing tremor and heat intolerance For the last e weeks she had experienced tachycardia at minimal exercise increasing weakness fatigue and nervousness During the last two weeks small red spots had appeared on her legs and lower abdomen Her current menstruation had been in progress for five days Her previous bleedings were regular lasting 5–6 days with intervals of 28 days and characterized as moderately increased She had not taken any drugs before entry

On admission she appeared very pale with numerous petechiae on the lower parts of the body She was obvi ously thyrotoxic with smooth moist warm skin tremor of the fingers and a hyperactive general appearance No exophthalmus was noted The thyroid gland was diffusely enlarged and a grade 3/6 systolic murmur was audible over the gland The spleen was not palpable A continuous vaginal bleeding was observed The pulse was 128 tem perature 37 9°C and blood pressure 155/75

Laboratory findings. Hb 42 g/l MCV 74 fl MCHC 30 g/l WBC 4 1×10⁹/l with a normal differential count platelet count 28×10⁹/l reticulocytes 8 0–3 8% Serum bilirubin was elevated 66–35 μmol/l (upper normal limit 21) and haptoglobin was low 0 06–0 08 g/l Serum iron was 4 μmol/l (lower normal limit 10) and TIBC 61 μmol/l Serum cholesterol was 2.2 mmol/l thyroxine 200 nmol/l T_3-sephadex 183% The ^{131}I uptake was 46% after 2 hours and 68% after 24 hours Fine needle aspiration biopsy of the thyroid gland showed focal lymphoid thyroiditis Autoantibodies against thyroid cell surface antigens studied by mixed haemabsorption technique showed a titre of 1/6 140 with a ring zone reaction Sternal bone marrow ex amination showed a highly active erythropoiesis and an increased number of megakaryocytes There were no bone marrow haemosiderin or sideroblasts The spleen was normal sized on flat X ray examination The mean life span of ^{51}Cr tagged thrombocytes was extremely shortened 0 02 days compared to normal (6 9±0.3) (Fig. 1) (5)

The clinical diagnosis was thyrotoxicosis with haemolytic anaemia in combination with anaemia due to blood losses and ITP

As shown in Fig 2 the platelet count fell to very low values within the first few days and the menorrhagia in creased in intensity The anaemia was easily corrected with blood transfusions However her bleeding state and hyperthyroidism had to be managed The platelet count remained below 10×10⁹/l despite continuous infusion of 400 mg hydrocortisone/day and the danger of a bleeding catastrophy was imminent Since antithyroid drugs might further aggravate the thrombocytopenia it seemed unwise to use them at this stage Radioiodine therapy would have no effect in the acute phase Therefore corticosteroids and β-blocking agents were used with an obvious ameliora tion of her thyrotoxic symptoms in agreement with previ ous experience (17 34 36) When the patient s thyrotox icosis was under control it was decided to undertake splenectomy

The splenectomy was performed on the 11th day in hospital Epinephrine infusion and platelet concentrates were given during splenectomy as shown in Fig 3 The amount of blood lost during the operation was estimated at 150 ml The weight of the spleen was 120 g. During the two weeks following splenectomy platelet counts rose to values around 100×10⁹/l A study performed with ^{51}Cr labelled autologous platelets one month after the opera

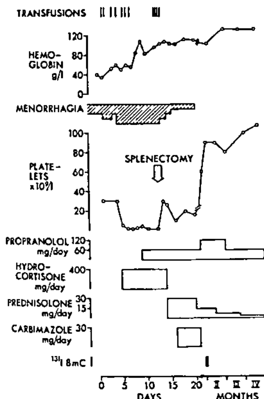


Fig 2 Course of the disease and treatment in case I

tion showed a platelet survival time of 6 2 days (normal range 4 5–8 8) compared to 0 02 days before splenectomy (Fig 1) Eight months after splenectomy platelet counts were 151×10⁹/l and the platelet mean life span (MLS) was 7 6 days In the meantime the patient had been treated with [131]iodine and she was euthyroid and in perfect condition when the second platelet survival study was performed Hb was 148 g/l and reticulocyte counts haptoglobin and bilirubin values were normal

Case 2

A woman aged 48 complained of tiredness dyspnoea on effort, palpitations heat intolerance nervousness and excessive sweating On examination there was fine finger tremor warm moist skin and a tachycardia of 106 beats/min The thyroid was diffusely enlarged and firm the left lobe somewhat larger than the right

Laboratory findings Hb 89 g/l WBC 5 2×10⁹/l with a normal differential count platelets 35×10⁹/l reticulocytes 5 0–1 6% Total serum bilirubin was 80–42 μmol/l 71–36 μmol/l of this amount was not conjugated CoHb 2 7% Triiodothyronine was highly elevated 9 1 nmol/l thyroxine 235 nmol/l The [131]iodine uptake in the thyroid was also elevated 36% after 2 hours and 69% after 24 hours Fine needle aspiration biopsy of the thyroid gland showed focal lymphoid thyroiditis Sternal bone marrow examination showed a hypercellular bone marrow (cellularity 90%) and a highly active erythropoiesis (52 6% erythroblasts) There were 61% sideroblasts and bone marrow haemosiderin grade II–III Bone marrow sections showed an increased number of megakaryocytes The spleen was normal sized on X ray examination and [99m]Tc scintigraphy The MLS of [51]Cr labelled platelets was extremely short (about 20 min) (Fig 1) Coombs direct and indirect tests were negative but there was a high titre of antibodies of the cold agglutinin type

The diagnosis was haemolytic anaemia and thrombocytopenia in a thyrotoxic woman She was initially given a β blocking agent (metoprolol) which partly controlled the thyrotoxic symptoms On day 14 after admission 6 0 mCi [131]I was given per os On this day her platelet count was 67×10⁹/l Seven days later platelet counts had decreased to 3×10⁹/l She had numerous petechiae and ecchymoses on her legs and abdomen During treatment with prednisolone (1 5 mg/kg b wt) platelet counts rose to 20–30×10⁹/l initially but then decreased on the same steroid dosage to values of 3–10×10⁹/l and remained at that level until splenectomy During corticosteroid therapy Hb rose to 100–115 g/l

On the day of splenectomy two months after treatment with [131]I Hb was 114 g/l and platelet counts were 6×10⁹/l Epinephrine and platelet concentrates were given in connection with splenectomy as shown in Fig 3 The spleen weighed 85 g After splenectomy platelet counts gradually rose to values around 100×10⁹/l and Hb to 130 g/l Seven months after admission and four months after splenectomy Hb was 138 g/l WBC 8 5×10⁹/l and platelets 133×10⁹/l At this time the mean platelet survival was 4 9 days (Fig 1) The patient was euthyroid in good health and had no complaints

DISCUSSION

It is generally accepted that in addition to supportive measures such as prevention of trauma and careful medical supervision the institution of high doses of corticosteroids should be the first choice of specific treatment in patients with acute ITP with low platelet counts and bleeding manifestations (3 10) Corticosteroids appear to exert their influence by decreasing the destruction of already sensitized platelets (30) and by reducing the titre of antiplatelet factor (15) As a result of these two effects platelet survival is in most cases prolonged resulting in an increase in the peripheral platelet count (6) In our two patients however administration of corticosteroids did not influence the peripheral platelet count which in both patients remained at a level of 3–6×10⁹ platelets/l

Previous experience in the treatment of a large series comprising 110 cases of patients with ITP has shown that in a substantial percentage (28%) of patients with acute ITP there was no increase in the peripheral platelet count even when very high doses of corticosteroids were given (7) The cases with

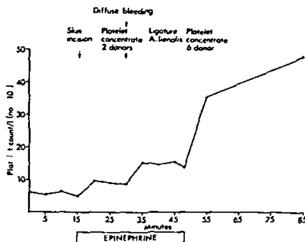


Fig 3 Venous platelet count in relation to different steps during splenectomy Epinephrine infusion is started 1 min before skin incision and is continued until ligation of the splenic artery By this procedure most of the platelets harboured in the splenic platelet pool are expelled into the circulation and transfused platelets are prevented from entering the spleen As seen there was a slight increase in the peripheral platelet count after the start of epinephrine infusion About 20 min after the beginning of the operation the patient began to bleed diffusely from wound surfaces and peritoneum and at this time platelet concentrate from two donors was given This measure resulted in an increase in the platelet count to about 15×10^9/l and an apparent decrease in the bleeding tendency After ligation of the splenic artery the epinephrine infusion was discontinued and platelets from 6 donors were given These platelets can be expected to give a good haemostatic effect as there is no pooling and no platelet destruction after the ligation of the splenic artery As may be apparent this measure is both effective and lasting

acute ITP not responding to corticosteroids as well as the present two cases had as a common denominator very low peripheral platelet counts and an extremely short survival of ^{51}Cr labelled platelets One might suggest that a high level of antiplatelet antibodies or a special type of antibody that is very injurious" to the platelets is present in these cases of acute ITP

Corticosteroid doses of the magnitude given in acute ITP (1 5–2 5 mg prednisolone/kg b wt) cannot be administered for any length of time Therefore if the patient is symptomatic and there is no increase in peripheral platelet count during corticosteroid therapy splenectomy will have to be considered The crucial point is when to regard corticosteroid treatment as of no further use Complications after splenectomy especially infections increase appreciably if corticosteroids are maintained at high doses for more than 14 days (22) Mainly because of this it appears advisable to splenectomize symptomatic adult ITP patients with very low platelet counts after 14 days of high-dose prednisolone treatment (1 5–2 5 mg/kg b wt) if there is no rise in the peripheral platelet count If the patient is bleeding much and the risk of life threatening symptoms is imminent the operation has to be performed earlier

In order to diminish the risks of bleeding during splenectomy of severely thrombocytopenic ITP patients a procedure was developed which minimizes the bleeding tendency It includes administration of platelet concentrates and i v epinephrine infusion as shown in Fig 3 The epinephrine infusion is started just before the skin incision and continued until the ligation of the splenic artery This procedure expels most of the platelets harboured in the splenic pool which normally consists of about one third of the total body platelet number in splenomegaly even more (8) Transfusion of platelet concentrates is if possible postponed until the ligation of the splenic artery since transfused platelets given earlier may be rapidly destroyed If the bleeding tendency is highly elevated with diffuse bleeding from peritoneum and wound surfaces platelet concentrates from two blood donors giving 450 ml blood each and prepared with the aid of plastic bags (CDR 1331 Travenol Laboratories) are given earlier After ligation of the splenic artery platelet concentrates prepared from 4–8 donors are given These platelets can be considered to be efficient and of lasting effect since at that time there is no splenic pooling and no premature splenic destruction Earlier when these precautions were not taken the surgeon sometimes had to terminate the operation shortly after the skin incision because increased bleeding tendency made it too dangerous to continue With the above measures patients with extremely low platelet counts have been splenectomized without blood losses greater than anticipated in subjects with normal platelet counts undergoing an operation of that order

Our patient 1 was splenectomized after about 14 days of corticosteroid treatment At that time the hyperthyroidism was partly controlled by the combined effects of corticosteroids and propranolol and the patient was clinically in a good condition although still with vaginal bleedings Patient 2 was first treated for the hyperthyroidism initially with metoprolol and subsequently with the addition of

odine. Not until 3 *months* after dismission during which time she received varying doses of corticosteroids, was she *referred to the* Surgical Department for splenectomy.

Splenectomy eliminates a major site for cell destruction (30) and the place where *in most cases* a substantial antibody production *is present* (0). These two mechanisms probably also account for the beneficial effects of *splenectomy* in haemolytic anaemia. Platelet kinetics *is affected* in such a way that platelet survival *is substantially* prolonged after the operation (Fig. 1) (4). *Platelet* production which before *splenectomy is elevated* due to bone marrow stimulation *by the low* peripheral platelet count (5) *remains* higher than in controls *in the months af*ter splenectomy because of a sluggish *feed back*. These effects together *with the* removal of the splenic platelet *pool* by splenectomy result in most ITP cases in a *peripheral* platelet count significantly above normal *one month* postsplenectomy (4). In case 1 the *platelet count* rose rapidly (within two weeks) *after splenectomy* to values around 100×10 platelets/l. *In case 2* the platelet counts did not exceed 100×10⁹ platelets/l until two months after the *operation*. At this time the degree of haemolysis was *correspond*ingly reduced. Thus the increase in *the* peripheral platelet *count* in *these two* patients was slower and *not* as high as is usually seen after splenectomy *in uncomplicated* ITP (4).

The combi*nation of* ITP, haemolytic anaemia and hyperthy*roidism* is of great theoretical interest with regard to *a possible* common etiology. In cases of ITP *without* previous history of recent virus infection or *ingestion* of drugs prior to the appearance of low *platelet* levels, several points strongly favour an autoimmune basis for the development of thrombocytopenia (10).

Concerning the hyperthyroidism it was noted long ago that transient thyrotoxic symptoms were frequently found at the onset of lymphadenoid goitre (33) and that about 10% of thyrotoxic patients followed for 10–15 years gradually became myxoedematous (11), which seems to be the usual endpo*int* of extensive thyroiditis. Since then a number of authors have stressed the association of thy*rotox*icosis and thyroiditis (9, 6, 27). In the present two patients fine needle aspiration biopsies showed focal lymphoid thyroiditis and in patient 1 *in* whom serum was analyzed for thyroid antibodies a high titre was found. Thus it is very p*obable* that thyroiditis provides the basis for the hyp thy o d m n tl c tw p t d ahed In ly pl d thy o d t nt b l c g n t exc l c n onen f the h t n thy l l c been f nd tl *globul n collo d otl er th thy gl bul n* m *on l nt g n a* lear o p c t d hy r p n TSH) receptor) (35) Il re l w no rela n h p l ect cc t l l dy t l c l *l* d le n nd t v t cl ved th t the t th d n b d e only pl y t l d te role t tl development f the thyroid le t i thy d s 3 Recent stud c ind c te te d th t tl c bas c abn m l ty n thy d ti cor t ed to cell med ated n mune re ct ons According t th v ew defect ve mmunolog c surve ll nce (*specific de* fect *n* suppres or T lymphocyte) permits the surv val of clones of rg t p cif c elf re ct e T lymphocytes aris ng by random n t t on wh ch would nduce he thyro d les on t thyro d t s (31 35) These findings re co s tent w th the occur rence of thyro d t n genet cally pred posed p pu lat ons rather than n rel t on to ny k nd of thyro d njury caused by env ronment l f ctors uch s v ral d sea es g v ng changed compo t *on of* thyro d ant gen c det m nants or c c pe *of ant gen* from ecluded foll cles ag nst *wh ch there* w s no prev ous tolerance.

Furthermore T lymphocytes have the known property of st mulat ng and cooper t ng w th groups of B lymphocytes wh ch n con eq ence produce mmunoglobul ns (29). The appe r nce of al no n l clones of T lymphocytes d rected g n t no n l self const tuents w uld in th s w y expl n tl c p es ence of autoant bod es found n lymph d tl y ro d t s. One of the ant bod es however h s n effect wh ch s un que for thy o d d c c e the one d ected towards ecep r n the tl y d cell membrane. Th s ant body once att ch ed t tl c TSH receptor appe rs namely t *I ve the prop* erty of *l l* k the thyro d cells (24 35). *It* is mechan sm m ght expl n *tl e thyr t x c* yn p s n thyro d t.

The les ons *n thy d t s* tl c s ce t t l c t duced m nly by cell *med ated* u c c cl s wh le in ITI there s ev dence tl t t l *l (4) f* the cases platelet de truct n is due t *nt pl telet* ant bod es (1() Tl e m st nte c t g *pe t n tl c* p c ent tw ca es is the p esence f tl c e tw types f mmunol g c ce l de truct n t ne d tl e v c nd v dual. If o e s l ok ng f r t k n m nator tl ere n st c tl er l c c ses w l *ITI* wh m p emat t c platelet destr *t*

cell mediated mechanisms or subjects with thyroid disease in whom the antibodies produced are injurious to platelets Of these two mechanisms the former seems to be the more probable Previous studies have shown that plasma from only 60% of ITP patients produces thrombocytopenia when transfused to normal recipients and that antiplatelet antibodies can be found by means of in vitro methods in at most 65% of patients with ITP (16 19) Thus in 35–40% of ITP patients other ways of platelet destruction than by antibodies seem to be present

The two patients described here behaved somewhat differently to the majority of cases with ITP since they did not respond with an increase in platelet counts after administration of high doses of corticosteroids and the rise in platelet levels after splenectomy was slower and less pronounced than is usually seen (4) The appearance of T lymphocytes directed against normal self constituents could explain both the thyroid lesions and the increased destruction of platelets and probably also the premature elimination of red cells as was demonstrated by the presence of haemolytic anaemia in our two patients The beneficial effects of splenectomy in these two cases could be explained by diminished contact between circulating platelets and immunocompetent cells since due to the circulation of blood through the spleen there a closer contact between immunocytes and cir ating corpuscles and various antigens in the spleen than in any other organ (32) Finally it is suggested that the association of thrombocytopenia and thyroid disease may not be all that rare since we found some kind of thyroid disease in 12% of our 110 ITP patients (7) (usually substituted for previously diagnosed thyroid hypofunction)

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REFERENCES

1 Aster R H Pooling of platelets in the spleen role in the pathogenesis of hypersplenic thrombocytopenia J Clin Invest 45 645 1966
2 Aster R H & Keene W R Sites of platelet destruction in idiopathic thrombocytopenic purpura Br J Haematol 16 61 1969
3 Baldini M Idiopathic thrombocytopenic purpura and the ITP syndrome Med Clin North Am 56 47 1972
4 Branehog I Platelet kinetics in idiopathic thrombocytopenic purpura (ITP) before and at different times after splenectomy Br J Haematol 29 413 1975
5 Branehog I Kutti J & Weinfeld A Platelet survival and platelet production in idiopathic thrombocytopenic purpura (ITP) Br J Haematol 27 127 1974
6 Branehog I & Weinfeld A Platelet survival and platelet destruction in idiopathic thrombocytopenic purpura before and during treatment with corticosteroids Scand J Haematol 12 69 1974
7 — Unpublished data
8 Branehog I Weinfeld A & Roos B The exchangeable splenic platelet pool studied with epinephrine infusion in idiopathic thrombocytopenic purpura and in patients with splenomegaly Br J Haematol 25 239 1973
9 Buchanan W W Alexander W D Crooks J Koutras A A Wayne E J Andersson J R & Goudie R B Association of thyrotoxicosis and autoimmune thyroiditis Br Med J 1 843 1961
10 Dameshek W ITP Scand J Haematol (Suppl) 9 61 1965
11 Eason J Correlation of Grave s disease and thyroiditis Edinburgh Med J 35 169 1928
12 Epstein R D Lozner E L Coffey T S & Davidson C S Congenital thrombocytopenic purpura Purpura haemorrhagica in pregnancy and in the newborn Am J Med 9 44 1950
13 Evans R S Takahashi K Duane R T Payne R & Liu C H Primary thrombocytopenic purpura and acquired hemolytic anemia evidence for a common etiology Arch Intern Med 87 48 1951
14 Finochietto R & DelCastillo E B Bocio exoftálmico y purpura hemorragica trombocitopenica Sem Med 43 1059 1936
15 Harrington W J Minnich V & Arimura G The autoimmune thrombocytopenias In Progress in hematology (ed L M Tocantins) vol 1 pp 166–192 Grune & Stratton New York 1956
16 Harrington W J Minnich V Hollingsworth J W & Moore C V Demonstration of a thrombocytopenic factor in the blood of patients with thrombocytopenic purpura J Lab Clin Med 38 1 1951
17 Howitt G & Rowlands D J Beta sympathetic blockade in hyperthyroidism Lancet 1 628 1966
18 Jackson A S Acute hemorrhagic purpura associated with exophthalmic goiter JAMA 96 38 1931
19 Karpatkin S Strick N Karpatkin M B & Siskind G W Cumulative experience in the detection of antiplatelet antibody in 234 patients with idiopathic thrombocytopenic purpura systemic lupus erythematosus and other clinical disorders Am J Med 52 776 1972
20 Karpatkin S Strick N & Siskind G W Detection of splenic antiplatelet antibody synthesis in idiopathic

autoimmune thrombocytopenic purpura (ATP) Br J Haematol 23 167 1972
21 Lamberg B A Kivikangas V Pelkonen R & Vuopio P Thrombocytopenia and decreased life span of thrombocytes in hyperthyroidism Am Clin Res 3 98 1971
22 Macpherson A I S & Richmond J Planned splenectomy in treatment of idiopathic thrombocytopenic purpura Br Med J 1 64 1975
23 Marshall J S Weisberger A S Levy R P & Breckenbridge R T Coexistent idiopathic thrombocytopenic purpura and hyperthyroidism Ann Intern Med 67 411 1967
24 Mukhtar E D Smith B R Pyle G A Hall R & Vice P Relation of thyroid stimulating immunoglobulins to thyroid function and the effects of surgery radioiodine and antithyroid drugs Lancet 1 713 1975
25 Najaean Y Ardaillou N Dresch C & Bernard J The platelet destruction site in thrombocytopenic purpuras Br J Haematol 13 409 1967
26 Papapetrou P D & Jackson I M D Thyrotoxicosis due to silent thyroiditis Lancet 1 361 1975
27 Peake L R Thyroiditis Postgrad Med 57 95 1975
28 Robson H N & Davidson L S P Purpura in pregnancy with special reference to idiopathic thrombocytopenic purpura Lancet 2 164 1950
29 Roitt I M Greaves M F Torrigiani G Brostoff J & Playfair J H L The cellular basis of immunological responses a synthesis of some current reviews Lancet 2 367 1969
30 Shulman N R Weinrach R S Libre E P & Andrews H L Role of reticuloendothelial system in pathogenesis of idiopathic and thrombocytopenic purpura Trans Assoc Am Physicians 78 374 1965
31 Siegal F P Suppressors in the network of immunity N Engl J Med 298 102 1978
32 Tavassoli M & Weiss L Splenic sickling In Formation and destruction of blood cells (ed T J Greenwalt & G A Jamieson) pp 108–174 Lippincott Philadelphia 1970
33 Vaux D M Lymphadenoid goitre Study of 38 cases J Pathol 46 441 1938
34 Vink A I Pimstone B L & Hoffenberg R Sympathetic nervous system blocking in hyperthyroidism J Clin Endocrinol 8 725 1968
35 Volpe R The role of autoimmunity in hypoendocrine and hyperendocrine function Ann Intern Med 87 86 1977
36 Werner S C & Platman S R Remission of hyperthyroidism (Graves disease) and altered pattern of serum thyroxine binding induced by prednisone Lancet 2 751 1965

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Fatal Renal Vasculitis and Minimal Change Glomerulonephritis Complicating Treatment with Penicillamine

Report on Two Cases

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ABSTRACT Two cases with different and not previously described fatal renal complications during treatment with penicillamine are reported A man with seronegative rheumatoid arthritis with features of systemic lupus erythematosus was treated with penicillamine for six months and developed a mild membranous glomerulonephritis and a severe renal vasculitis leading to uremia and death A woman with primary biliary cirrhosis was treated with penicillamine for nine months and developed a nephrotic syndrome, the renal biopsy showing minimal change glomerulonephritis The nephrotic syndrome responded to prednisone but the patient died, probably from septicemia Penicillamine may thus cause glomerular damage without deposition of immune complexes A restricted use of the drug is recommended

Key words penicillamine vasculitis nephrosis
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For more than ten years penicillamine has been used in the treatment of a variety of diseases including Wilson s disease cystinuria heavy metal poisoning scleroderma rheumatoid arthritis chronic aggressive hepatitis primary biliary cirrhosis and macroglobulinemia Renal complications are among the most serious of the adverse effects of the drug The incidence of penicillamine induced nephropathy varies between 0 and 17% in different series (2 19) In most of the cases reported the nephropathy has been membranous glomerulonephritis appearing clinically as a nephrotic syndrome which usually has been reversible after discontinuation of the drug (1 4 6 10 11) A more serious form of renal lesion has also been reported in a few cases with crescenteric proliferative glomerulonephritis leading to uremia and death (14 24)

We wish to report on two cases with fatal renal complications while on penicillamine both with unusual histological changes in the kidneys

CASE REPORTS

Case 1

A 56-year-old man had renal tuberculosis in 1963 This left the right kidney with a slight reduction of parenchyma though the overall renal function tests and urinalyses remained normal In 1971 the patient developed polyarthritis affecting the joints of the hands wrists elbows feet ankles and knees and he was admitted to the Rheumatism Foundation Hospital Heinola Finland The tests were negative for rheumatoid factor but positive for antinuclear factor (ANF) the titer being 1 1000 on average

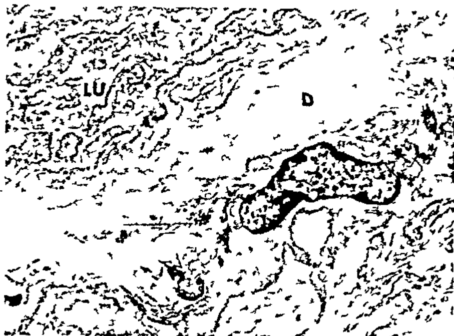
After four years small erosions were detected radiologically in some metatarsophalangeal joints No signs of systemic lupus erythematosus (SLE) other than the polyarthritis and the ANF titer could be detected The patient was considered to be suffering from seronegative rheumatoid arthritis with some features of SLE

Prednisone was given almost constantly from the onset of the disease in 1971 In March 1976 treatment with penicillamine 600 mg/day was started and the condition of the joints improved somewhat during the following months The serum creatinine and urinalysis were normal at the beginning of this treatment but after six months the patient was admitted to the Fourth Department of Medicine Helsinki University Central Hospital because of proteinuria 3 6 g/day Serum creatinine was then 178 μmol/l but serum albumin was normal and no edema was noted The treatment with penicillamine was stopped but prednisone treatment in a dose of 15 mg/day was continued However the renal function deteriorated rapidly and the patient was uremic in three months from the onset of proteinuria A needle biopsy of the left kidney was performed at this stage the serum creatinine being 1325 μmol/l The uremia was terminal and the patient was treated with peritoneal dialysis until he died from pneumonia four weeks later

Abbreviations SLE—systemic lupus erythematosus ANF—antinuclear factor GBM—glomerular basement membrane



Fg 1a and b Pat ent 1 L ght m crographs of necro t c and occluded preglomeru lar arteriole (A) hown n two compan on sect ons G tangent ally cut glomerulus Mas on s tri chrome ×3 0



Fg 2 Patient 1 Electron micrograph of preglomerular arteriole A depos t (D) of homogeneous f nely granular material is located subendothelially LU lumen oc cluded by f brillary material EI = internal ela t c lamina ×5300

Patholog cal f nd ngs

The renal b opsy was stud ed by light electron and immunofluorescence microscopy us ng standard techniques Light microscop cally the arterioles showed thickening of the wall with infiltration of various kinds of inflammatory cells and often f brinoid necros s of all layers of the wall with occlus on of the lumen (Fg. 1a and b) The necroses sometimes extended into the hilar region of the glomeruli such glomeruli showed segmental proliferation and ne croses of the tuft The majority of the glomeruli how e er showed only sl ght to moderate diffuse condensat on of the capillary tuft with wrinkling and tortuos ty of the glomerular basement membrane (GBM) i e alterations interpreted as due to ischemia. No th cken ng or spikes of the GBM ind cative of membranous glomerulonephritis were observable The med um-sized and large arteries appeared normal The tubules were atroph c and often plugged by hyaline casts The moderately edematous and f brotic nterst tium showed focal nfiltrates of inflamma tory cells that were usually concentrated around damaged arterioles and glomeruli

One damaged arteriole was present in the spec mens studied by electron microscopy Its luminar port on was occluded by cellular debris ntermingled with un dent f able granular and f brillary material One large depos t of homogeneous f nely granular material was present subendothelially (F g 2) The glomeruli showed part al or complete occlusion of capillary lumina due to swelling of

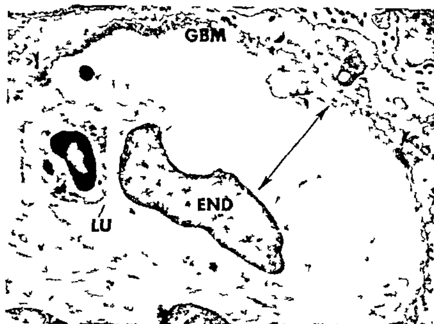


Fig 3 Patient 1 Electron micrograph of glomerular capillary showing isolated subepithelial deposits (arrowheads) The lumen (*LU*) is narrowed due to endothelial cell (*END*) hyperplasia and pronounced swelling of the internal part (arrow) of the GBM ×7400

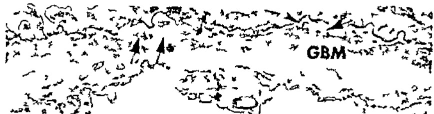


Fig 4 Patient 1 Electron micrograph of a segment of the glomerular capillary wall showing multiple subepithelial deposits (arrows) surrounded by small projections (arrowhead) of the GBM The internal part of the GBM is broadened and electron lucent ×14 600

endothelial cell cytoplasm and pronounced broadening of the internal part of the GBM (Fig 3) Very sparse electron dense deposits sometimes surrounded by small projections of the GBM were present along the epithelial surface of the GBM (Figs 3 and 4) a finding consistent with mild membranous glomerulonephritis

On immunofluorescence the glomeruli showed diffuse granular deposits of IgG and C3 along the capillary walls (antisera against IgG IgA IgM C3 and fibrin were used) (Fig 5) No arterioles or larger arteries were present in the specimen studied by immunofluorescence

At autopsy no vasculitis alterations could be detected in any organ other than the kidneys Bronchopneumonia was considered to be the immediate cause of death

Case 2

A 57 year old woman was found to have a high ESR and the diagnosis of primary biliary cirrhosis was confirmed in 1972 by liver biopsies and by the detection of a high titer of antimitochondrial antibodies in serum In 1973 prednisone and azathioprine were given for some months

Treatment with penicillamine 450 mg/day was started in Feb 1976 At this time the serum creatinine was normal and no urinary abnormalities were present The penicillamine dose was increased to 600 mg/day after five months of therapy and four months later the patient began to develop lower limb edema and proteinuria She was also found to have an E coli urinary tract infection and was given furosemide and cephalexine by a district physician

On admission to Helsinki University Central Hospital five days later the patient had pitting edema up to the level of the knees and bilateral pleural effusion Urinalyses revealed proteinuria 2 7 g/l and the urinary tract infection but no hematuria Serum creatinine was 198 µmol/l and serum albumin 10 8 g/l The treatment with penicillamine was discontinued but in spite of large doses of furosemide the edema persisted the diuresis did not exceed 500 ml and the serum creatinine rose to 398 µmol/l within ten days of admission Prednisone 75 mg/day was given on the tenth day The diuresis increased promptly the serum creatinine normalized within six days and the edema subsided Renal needle biopsy was performed on the 12th day

On the 17th day while still on prednisone the patient developed large petechiae and anuria and died within a few hours from acute circulatory failure probably due to septicemia The cause of death remains uncertain since no blood cultures had been made but the presumptive septicemia was supported by the autopsy findings



Fig 5 Patient 1 Fluorescent micrograph of a glomerulus stained with anti human IgG Diffuse granular deposits of IgG are present along capillary walls ×470

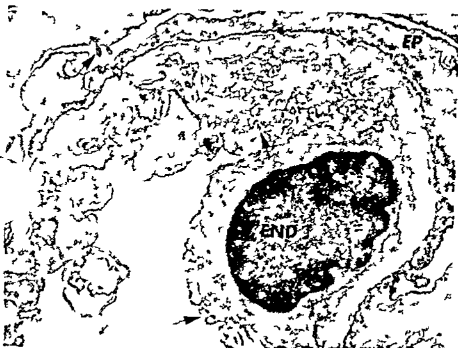


Fig 6 Patient 2 Electron micrograph of glomerular capillary The foot processes of epithelial cells (EP) are diffusely lost Both epithelial and endothelial (END) cells show microvillus formation (arrows) Deposits are lacking ×11 100

Pathological findings

The renal biopsy showed normal glomeruli by light microscopy The tubular epithelium contained hyaline droplets indicative of heavy proteinuria The interstitium and blood vessels were normal

On electron microscopy the glomeruli showed alterations typical of minimal change glomerulonephritis i e complete loss of the foot processes of epithelial cells epithelial and endothelial cell microvillus formation endothelial cell swelling and lucent broadening of the internal part of the GBM (Fig 6) No electron-dense deposits were observed

The immunofluorescence staining of glomeruli was negative

At autopsy the renal glomeruli showed diffuse intravascular coagulation A few glomeruli contained masses of bacilliform bacteria thus indicating terminal septicemia with associated intravascular coagulation Microvascular thrombi with perivascular bleeding were also present in skin specimens with macroscopically s

ble petechiae Micronodular cirrhosis of the liver was confirmed showing disappearance of bile ducts and thus corresponding to a late stage of primary biliary cirrhosis

DISCUSSION

The two patients developed their fatal renal complications after six and nine months of penicillamine therapy respectively It has repeatedly been reported that most cases of penicillamine induced nephropathy occur between five months and one year from the beginning of treatment (1 4 6 9 10 19) the extremes being four weeks (8) and six years (25)

In the series of penicillamine induced nephropathy reported so far the biopsy findings usually have been those of membranous glomerulonephritis as studied either by light electron or immunofluorescence microscopy (1 4 6 10 11) Only one case with normal findings on light electron and immunofluorescence microscopy has been reported (9) Another type of renal lesion has also been seen in some cases with crescenteric proliferative glomerulonephritis (14 24) In these cases too the immunofluorescence showed granular deposits of IgG and C3 (24) and electron microscopy showed electron-dense subepithelial deposits (14) These findings indicate that the pathogenesis of penicillamine induced nephropathy is due to immune complex deposition in the glomeruli and this seems to be true of both the crescenteric proliferative and the membranous variants of glomerulonephritis

The cases with membranous glomerulonephritis appearing clinically as proteinuria or as a nephrotic syndrome have usually been reversible after discontinuation of the drug although at least some degree of proteinuria can persist for 12 months or more (1 6) The cases with crescenteric proliferative glomerulonephritis have presented a picture resembling Goodpasture s syndrome and progressed to uremia and death (14 24) One patient with Wilson s disease died from anuria while on penicillamine but no pathological findings were reported (28) and one patient with systemic sclerosis died from acute renal failure while on penicillamine (27)

Our patient 1 developed a morphologically mild membranous glomerulonephritis and a severe vasculitis the latter being confined to the kidneys The finding of membranous glomerulonephritis evident on electron and immunofluorescence microscopy conforms to findings in previous reports and is consistent with an immune complex pathogenesis However the vasculitis dominated the histologic picture and was undoubtedly responsible for the renal failure The presence of an electron-dense deposit in the wall of a damaged arteriole may indicate that the vasculitis was also mediated by immune complexes Consequently the drug could have initiated the formation of both small and large complexes resulting in both glomerulitis and vasculitis in analogy with known experimental models (7) Unfortunately the specimen stained for immunofluorescence did not contain any vessels

The underlying disease in case 1 had features of both rheumatoid arthritis and SLE Several kinds of renal lesions are possible in rheumatoid arthritis (26) but vasculitis in the kidney is very uncommon in this disease even with extensive visceral vasculitis and when present it does not seem to occur in isolation from such a generalized vasculitis (12 15 21 23) Furthermore vasculitis seems to be a feature of classical seropositive rheumatoid arthritis (16) The kidneys are often affected in SLE but lupus nephritis always seems to take one of several forms of glomerulitis (3 5 22) A mild form of vasculitis may occasionally be seen in association with active lupus nephritis (18 20) but most authors do not even mention vascular lesions in their biopsy series On the basis of clinical and autopsy findings periarteritis nodosa Wegener s granulomatosis Henoch Schonlein s syndrome and other similar conditions could be ruled out as a cause of the vasculitis in our case 1 The patient did not have hypertension or hepatitis B either which can be associated with similar vascular lesions

Case 2 had normal light microscopic histology negative immunofluorescence staining and only fusion of the foot processes of the glomeruli on electron microscopy all these findings in conjunction with the nephrotic syndrome being typical of minimal change nephropathy Nephropathy of this type has not been reported to occur in association with primary biliary cirrhosis Neither has minimal change nephropathy been reported earlier to be penicillamine induced The only reported case with normal findings on light electron and immunofluorescence microscopy had only protein uria *2–3 g/l* and *no nephrotic syndrome* (9) The pathogenesis of minimal change nephropathy still remains obscure but immune complexes not considered to play any role in it (13) \

cell bound immunity is involved in the pathogenesis of this syndrome as has been suggested (17) and whether penicillamine could affect this immune system is still unknown Our case 2 shows that penicillamine seems to be able to produce a minimal change glomerulonephritis leading to nephrotic syndrome without immune complex deposition in the kidneys

Although there is a concentration of nephrological cases in our department we feel that the occurrence of two fatal renal complications during treatment with penicillamine within one year justifies us in recommending restricted use of the drug

REFERENCES

1 Bacon P A Tribe C R Mckenzie J C Verrier Jones J Cumming R H & Amer B Penicillamine nephropathy in rheumatoid arthritis Q J Med 180 661 1976

2 Berry H Liyanage S P Durance R A Barnes C G Berger L A & Evans S A controlled trial comparing azathioprine and penicillamine in the treatment of rheumatoid arthritis Br Med J 1 1052 1976

3 Cameron J S Turner D R Vosnides G Leibowitz S Lessof M H Ogg C S Chantler C & Brown C B The kidney in systemic lupus erythematosus In Seminars in nephrology p 41 Wiley New York 1977

4 Dische F E Swinson D R Hamilton E B D & Parsons V Immunopathology of penicillamine induced glomerular disease J Rheumatol 3 145 1976

5 Dubois E L Lupus erythematosus 2nd ed p 72 University of Southern California Press Los Angeles 1974

6 H V Neild G H Bohle A Hallauer W Hoppe Seyler G Luttgen F M & Scholl meyer P Perimembranose Glomerulonephritis nach Penicillamintherapie Klin Wochenschr 53 835 1975

7 Germuth F G Jr & Rodriquez E Immunopathology of the renal glomerulus p 163 Little Brown & Co Boston 1973

8 Haas P & Wendt H Nephrotisches Syndrom und Schilddrusenschwellung nach D-penicillamin Wien Med Wochenschr 22 333 1974

9 Huskisson E C & Dudley Hart F Penicillamine in the treatment of rheumatoid arthritis Ann Rheum Dis 31 402 1972

10 Jaffe I A Treser G Suzuki Y & Ehrenreich Th Nephropathy induced by D-penicillamine Ann Intern Med 69 549 1968

11 Lachmann P J Nephrotic syndrome from penicillamine Postgrad Med J (Suppl) 44 23 1968

12 Lawson A A H & Maclean N Renal disease and drug therapy in rheumatoid arthritis Ann Rheum Dis 25 441 1966

13 Mallick N P The pathogenesis of minimal change nephropathy Clin Nephrol 7 87 1977

14 McCormick J N Wood P & Bell D Penicillamine induced Goodpasture s syndrome In Penicillamine research in rheumatoid disease (ed E Munthe) p 268 Merck Sharp & Dohme symposium at Spå tind Norway March 1976

15 Meriwether J H Jr Weinberger H J & Gleason I O The renal vascular lesion in rheumatoid disease Arthritis Rheum 10 298 1967

16 Mongan E S Cass R M Jacox R F & Vaughan J H A study of the relation of seronegative and seropositive rheumatoid arthritis to each other and to necrotizing vasculitis Am J Med 47 23 1969

17 Moorthy A V Zimmerman S W & Burkholder P M Nephrotic syndrome in Hodgkin s disease Am J Med 61 471 1976

18 Muehrcke R C Kark R M Pirani C L & Pollak V E Lupus nephritis a clinical and pathological study based on renal biopsies Medicine 36 1 1957

19 Multicenter Trial Group Controlled trial of D-penicillamine in severe rheumatoid arthritis Lancet 1 275 1973

20 Rothfield N F McCluskey R T & Baldwin D S Renal disease in systemic lupus erythematosus N Engl J Med 269 537 1963

21 Ruddy S & Castleman B Cardiac and renal failure with rheumatoid arthritis N Engl J Med 285 1250 1971

22 Sinniah R & Feng P H Lupus nephritis correlation between light electron microscopic and immunofluorescent findings and renal function Clin Nephrol 6 340 1976

23 Sokoloff L & Bunim J J Vascular lesions in rheumatoid arthritis J Chron Dis 5 668 1957

24 Sternlieb I Bennett B & Scheinberg I H D-Penicillamine induced Goodpasture s syndrome in Wilson s disease Ann Intern Med 82 673 1975

25 Walshe J M Toxic reactions to penicillamine in patients with Wilson s disease Postgrad Med J (Suppl) 44 6 1968

26 Wegelius O Renal lesions in rheumatoid arthritis In Non articular forms of rheumatoid arthritis (ed T E W Feltkamp) p 192 Stafleu s Scientific Publishing Co Leyden 1977

27 White A G In Other case reports and discussion of adverse reactions to penicillamine Postgrad Med J (Suppl) 50 79 1974

28 Yonis I Z & Karp M Chelating agents in Wilson s disease Lancet 2 689 1963

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Primary Sclerosing Cholangitis Associated with Fibrosis of the Submandibular Glands and the Pancreas

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ABSTRACT A new syndrome of primary sclerosing cholangitis associated with fibrosis of the submandibular glands and the pancreas is described in a 43-year-old male The sclerosing cholangitis was diagnosed at laparotomy because of cholestasis and the fibrosis of the submandibular glands and pancreas confirmed at microscopical investigation of biopsy specimens The cholangitis responded well to treatment with a low dose of prednisolone (7 5–10 mg) and an endoscopic retrograde cholangiopancreaticographic examination 10 months after the operation revealed normal bile ducts

Primary sclerosing cholangitis is a rare disease characterized by a diffuse inflammatory thickening and fibrosis of the biliary ductal walls The process may affect the entire extrahepatic ductal system or a part of it Intrahepatic involvement is common Care should be taken not to mistake cholangiocarcinoma for primary sclerosing cholangitis since they have many clinical features in common (2 5)

Primary sclerosing cholangitis is often associated with ulcerative colitis but in several cases no apparent association with other diseases can be found A few cases have been reported in which an inflammatory reaction and fibrosis of other tissues co-existed such as Riedel s struma (1 3) retroperitoneal fibrosis (1 3) and inflammatory pseudotumour of the orbit (6) The sicca complex and chronic pancreatitis was recently reported in two siblings with primary sclerosing cholangitis (4)

The present paper describes the association of primary sclerosing cholangitis with fibrosis of the submandibular glands and the pancreas

CASE REPORT

The patient a male with a qualified office job aged 43 in 1976 was healthy until 1973 when he during the autumn noted swelling of the submandibular glands and for a few months blocked nose dry eyes and mouth Efforts to obtain material from the glands for cytological investigation failed repeatedly X ray revealed no duct stones In April 1974 diabetes mellitus was diagnosed and treatment was started with diet alone

One year later the patient felt tired and complained of slight gastric pain On examination both submandibular glands were enlarged and firm No epigastric mass or hepatomegaly was found Results of investigations were Hb 144 g/l ESR 20 mm/h WBC 5 1×10⁹/l Normal differential count on all occasions Serum bilirubin 40 μmol/l alkaline phosphatase 5 0 μkat/l Hb_sAg negative Normal levels of immunoglobulins and no autoantibodies In addition to slight steatosis a percutaneous liver biopsy specimen showed bile plugs in the canaliculi as a sign of cholangitis An X ray of the chest and a barium meal revealed normal findings The level of alkaline phosphatase increased further and a laparotomy in June 1975 revealed a dilated gallbladder containing no stones The liver appeared normal Most of the bile duct was changed into a hard string The head of the pancreas was firm and malignancy was suspected but a needle biopsy did not confirm this It showed however increased fibrosis Several enlarged lymph nodes were found in the mesenterium of the small intestine Histologically a non specific inflammatory reaction was seen A cholangiogram performed during operation indicated a total occlusion of the choledochus No attempt was made to by pass the obstruction surgically The patient was referred to the University Hospital in Uppsala for further treatment Corticosteroids were administered orally and the patient responded dramatically The bile ducts appeared normal at an i v cholangiogram two months later However his diabetes mellitus now required insulin treatment Since then (1 1/2 years) several attempts to decrease or withdraw prednisolone have been made but have resulted in recurrence of symptoms of bile duct obstruction (Fig 1) In April 1976 the bile duct was normal when examined

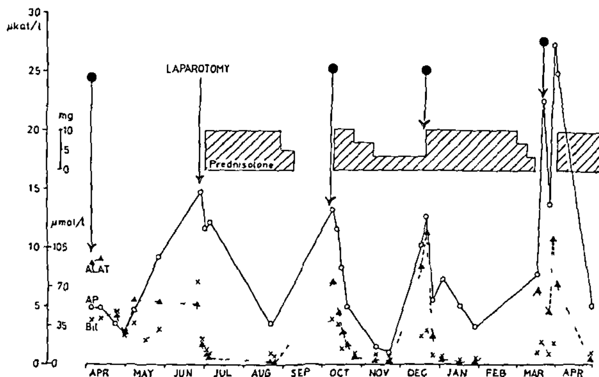


Fig 1 Symptoms including malaise itching clay coloured stools dark urine and frank icterus (●) recurred following reduction of the prednisolone dosage together with an increase of serum alkaline phosphatase (O—O) serum alanine aminotransferase (▲ ▲) and serum bilirubin (× ×)

endoscopic retrograde cholangiopancreaticography However only part of the pancreatic duct could be filled with contrast indicating a block in the midportion of pancreas Additional investigations in 1976 showed a decreased ... in output (Lundh test) although no steatorrhoea was ... An i v glucose tolerance test was abnormal ...ng a low insulin response The right sided sub...ular gland was explored and a specimen was taken ... a part of it seeming macroscopically normal another specimen was taken from the firm fibrotic portion In both specimens there were signs of an inflammatory reaction with marked fibrosis in the firm specimen The size of the submandibular glands has remained constant during the last three years (Fig 2) The patient has remained in good health since the start of the continuous prednisolone treatment and has been able to continue with his rather strenuous work

DISCUSSION

The investigations of the present case showed that the disease process involved not only the bile duct but also the pancreas and the submandibular glands In the analysis of Smith and Loe (3) of disorders associated with primary sclerosing cholangitis the increased incidence of pancreatitis observed was considered more than coincidental However to our knowledge no case of submandibular gland fibrosis has been described in association with primary sclerosing cholangitis

Whether the fibrosis of the affected glands primarily emerged from the ducts or the adenomatous tissue itself or was secondary to duct obstruction could not be properly evaluated However a direct involvement of the glandular tissue was indicated by the evidence obtained including an inflammatory reaction in one specimen of the submandibular glands and a patent duct in the head of the fibrotic pancreas This view was further underlined by the fact that the endocrine function of the pancreas was deranged at an early stage of the disease

The pathogenesis of primary sclerosing cholangitis is still unknown although various hypotheses have been discussed in the literature e g autoimmunity Increased levels of gammaglobulin (6) and blood eosinophils (1 4) have been observed in some cases The present patient had repeatedly normal eosinophil counts normal serum electrophoresis and no evidence of autoimmunity in additional tests However as illustrated in Fig 1 the response



F g 2 Front v ew of the neck The arrows nd cate the pos t on and s ze of the f brot c submand bular glands

to a low dose of prednisolone was excellent In some of these respects our pat ent d ffers from the two s blings described by Waldram et al (4) hav ng the sicca complex and chron c pancreat t s n as soc at on with primary scleros ng cholang t s but n other respects the sim lant es were striking Thus the two sibl ngs had swell ng of the submandibular glands although f brosis was not reported Further mo e they exh b ted s gns of nsuff c ent endo and exocrine pancreat c funct on s milar to those seen n our pat ent

It thus seems as f an nflammatory react on w th or w thout f bros s of the submand bular glands pancreas and poss bly the lacrimal glands (as nd cated by periods of dry eyes) may occur n assoc at on w th primary scleros ng cholang t s and form a d sease ent ty It s poss ble that this ent ty is one of the several ways in which the f brotic d sease can be exp essed However the ncreas ng number of d sease ent t es being described w h ch nclude primary sclerosing cholang tis m ght ind cate that there are several pathogenetic mechanisms beh nd the development of this ductal wall d sease

REFERENCES

1 Bartholomew L G Cain J C Woolner L B Utz D C & Ferris D O Scleros ng cholang t s Its pos s ble assoc at on w h R edel s struma and f brous re t operiton t s Report of four cases N Engl J Med 69 8 1963

2 Schwartz S I Primary scle os ng cholang s A d s ease re ed Su g Cl n North Am 53 1161 1973

3 Sm h M P & Loe R H Sclerus ng cholang s Rev ew of ecent ca e eports and assoc ated d seases and four new c ses Am J Surg 110 239 1965

4 Wald am R Kopelman H Tsantoulas D & W l l ans R Ch on c pancrea t s scleros ng cholang s and cca complex n two s b ngs Lancet 1 550 1975

5 Warren K W Athanas ades S & Monge J J Primary sc e o ng cholang s A s udy of f f y two cases Am J Su g 111 23 1966

6 Wenger J G ngrich G W & Mendeloff J Scleros ng cholang t s—a man festa on of sys em c d sease A ch In e n Med 116 509 1965

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Phasic Voltage Alternation in Spontaneous Left-Sided Pneumothorax

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ABSTRACT. Marked phasic voltage alternation is described in a man with spontaneous left-sided pneumothorax. This ECG change in association with pneumothorax has been described in only one patient previously.

Key words: pneumothorax, electrocardiography.
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Electrocardiographic changes occurring in association with pneumothorax are sometimes confusing for the diagnosis and direct attention away from the primary cause of the alternation. This has been reviewed recently by Walston et al. (4) and by Eriksson and Jorfeldt (1). In addition to these changes Kuntzky and Goldfarb (2) described a young woman with spontaneous pneumothorax associated with a marked phasic voltage alternation in the ECG together with a shift of the P, QRS and T axes. To the best of our knowledge, this has not been described further in the literature and it therefore seems justified to report a similar case.

CASE REPORT

A 37-year-old male, previously healthy, smoking 15 cigarettes daily, was admitted to our hospital because of left-sided chest pain and moderate dyspnoea. Six days earlier he had experienced slight pain in the left side of the chest upon breathing, but on the day of admission he felt worse, with constant pain and dyspnoea.

On physical examination it was noted that the heart sounds were more distinct on the right side of the sternum and a diminished breath sound over the left lung was present. Apart from this, the physical examination did not reveal any abnormalities. ECG showed regular sinus rhythm with a rate of 80 beats/min, a mean frontal plane QRS axis of about +70°, and a marked phasic voltage alternation, most apparent in leads II and V_1 (Fig. 1). Chest X-ray upon admission revealed a left-sided pneumothorax, with complete collapse of the left lung and with a deviation to the right of the mediastinum, most marked at expiration.

The condition of the patient required treatment with pleural suction, which was successfully carried out. On the tenth day of hospitalization he was discharged in good condition. Four days later, a control of the ECG showed sinus rhythm as before, but the mean frontal QRS axis had shifted to about +30°, without any signs of electrical alternation or other abnormalities (Fig. 2).

DISCUSSION

Kuntzky and Goldfarb (2) have discussed some possible explanations for this ECG change, as a change of the position of the heart secondary to the shift to the right of the mediastinum corresponding

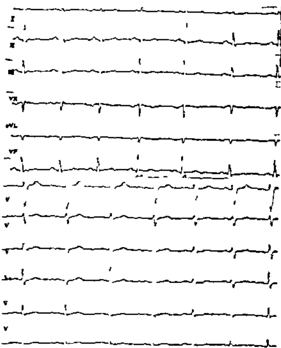


Fig. 1. ECG on admission showing marked phasic voltage alternation.

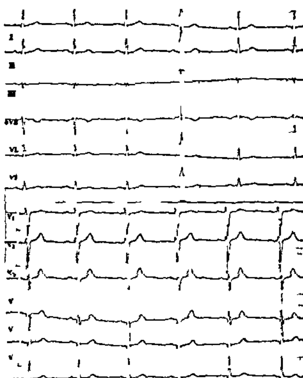


Fig 2 Normal ECG 2 weeks later

to the respiration Their case however presented with sinus tachycardia which in itself can cause electrical alternation (3) The case presented here further supports their hypothesis that the ECG changes can be attributed to the pneumothorax as such and that they are not secondary to the suddenly decreased respiratory function with subsequent tachycardia This conclusion implies that the ECG is a valuable tool in the diagnosis of pneumothorax though the exact explanation of the changes is still not known

REFERENCES

1 Eriksson S & Jorfeldt L EKG förändringar vid pneumothorax Läkartidningen 72 4395 1975
2 Kuritzky P & Goldfarb A L Unusual electrocardiographic changes in spontaneous pneumothorax Chest 70 535 1976
3 Schamroth L An introduction to electrocardiography p 167 Blackwell Scientific Publications Oxford and Edinburgh 1966
4 Walston A Brewer D L Kitchens C S & Krook J E The electrocardiographic manifestations of spontaneous left pneumothorax Ann Intern Med 80 375 1974

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REVIEW ARTICLE

Fetal Proteins in Viral Infections

The relation between viral infections and fetal protein synthesis is well documented in animals but less in humans Two types of associations are of interest *a*) viral proteins may behave like fetal antigens in that they have a high expression in fetal life and become reexpressed in adult life usually in connection with a tumor and *b*) fetal protein synthesis may follow a viral infection especially if the virus is oncogenic or displays a latent behavior

This article describes instances where associations have been noted between viral infections and fetal antigens The implications for tumor diagnosis will be discussed as well as possible functions of fetal antigens

Possible mechanisms for fetal protein occurrence in oncogenesis

Fetal antigens are expressed during embryonic life and in low quantities in healthy adult life High amounts are found in malignant tumors especially in organs which in the fetus are the principal sites of synthesis (5 7)

The renewed occurrence of these antigens in tumors might be an expression of already existent but silent genetic information Immunological identities between several fetal and tumor cell antigens strengthen the theory that the tumor product derives from derepressed fetal genes

Viruses induce new antigens on the surface of tumor cells The appearance of similar antigens within groups of tumors may have several causes such as the presence of endogenous or exogenous viruses

There are several examples of naturally occurring tumors caused by DNA viruses Experimentally tumors have been induced in animals by polyoma adeno- and SV40 virus and by partially inactivated herpes simplex and cytomegalovirus For example an SV40 induced tumor cell line has been transplanted in hamsters In these cells no infectious virus is synthesized but virus induced cellular antigens and fetal or phase specific antigens are produced (4) Immunization with fetal hamster tissue confers protection both to the transplantable cell line and to the tumor inducing effects of SV40 Consequently one of the SV40 surface antigens was called a carcinoembryonic antigen of the hamster (3)

Endogenous viruses of the RNA type are present in may species including humans Natural and experimental transmission by these or by exogenous C type RNA viruses has been shown to cause leukemias and sarcomas in animals

Endogenous viruses of the RNA type are transmitted as part of the genetic make up of infected animal cells Viral antigens are sometimes expressed in embryonic tissue in much the same fashion as fetal antigens (21) The occurrence of differing endogenous virogenes has provided arguments for the relationship between monkey species It also led to discussions on the Asian origin of man (2)

Fetal antigens in man

Human fetal antigens are not likely to derive from proper viral genes since no virus particles have been associated with the abundant occurrence of such antigens But fetal antigens may still be induced by viral or chemical mutagens Some antigens associated with human malignancies are expressed in the embryo and in tumor tissue in a manner similar to endogenous virus antigens in animals

Specific enzymes such as prostatic acid phosphatase in prostatic carcinomas monoclonal immunoglobulins in myelomas and ectopic human chorionic gonadotrophin (HCG) in choriocarcinomas have become important tools in diagnosing and monitoring tumors and are all highly specific for the tumor cells (24) HCG is also produced by the placenta during pregnancy There are several examples of the emergence of fetal proteins during neoplastic transformation Determination of fetal gene products is of immediate importance in management of tumors such as colorectal cancer (carcinoembryonic antigen CEA) hepatocellular carcinoma and testicular tumors (α

fetoprotein AFP) CEA and AFP are present in high concentrations in some fetal organs and in amniotic fluids

Germ cell tumors offer a potential for the study of many fetal antigens Malignant teratomas represent different stages of maturation of embryo like cells which also contain different antigens Several fetal antigens have been found in such tissue in different subpopulations of cells (23)

Large amounts of AFP are synthesized in *hepatoma* cells and raised serum concentrations are of clinical value to diagnose and monitor this dis ease AFP levels in serum are raised in healthy people from areas with a high risk of developing liver cancer compared to other areas

Infection with hepatitis B virus has been as sociated with the development of hepatomas (15) 90% of hepatoma patients have hepatitis B surface antigen in their sera and may have antibody to core antigen In families of patients with primary hepatic carcinoma the antigen distribution and antibody response to hepatitis B virus are more frequent than in matched control families

Several authors also have evidence suggesting that transiently raised AFP is directly associated with hepatitis B infections In virus infected moth ers and their children the AFP levels varied more than in non infected mothers perhaps due to liver affection

Primary cytomegalovirus (CMV) infections give transient liver damage and sometimes raised AFP in adults (6) These findings may indicate infection of certain liver cells or an indirect recruitment of AFP producing cells after virus infection

A rising level of serum CEA predicts recurrences after radical operation in around 70% of patients with *colon rectum* and *breast cancer* The lead time moreover often exceeds 6 months As with fetal antigens of animal tumors CEA is thought to represent a certain developmental stage of ento dermally derived cells The cellular CEA content is usually higher with more differentiated tumor cells In therapy resistant infections of long standing a substance immunologically similar to CEA is re leased Thus not only malignant but also normal human cells perhaps of a precursor cell type of the organ in question may release fetal antigens if properly stimulated

Recent evidence indicates that CEA may be a marker for an unidentified virus in non A non B hepatitis also called hepatitis C A support for this link between CEA and an infectious agent co[illegible] from Gitnick (17) who found that 62% of pati[illegible] with a non A non B hepatitis had raised C[illegible] levels and that transfusion of CEA positive bl[illegible] gave four times the frequency of hepatitis comp[illegible] to recipients of blood with normal CEA levels

In *leukemias* an abnormal proliferation and [illegible]ferentiation of white cells are seen Virus like p[illegible]cles and reverse transcriptase associated [illegible] particles having densities similar to mouse [illegible]kemias have been found in human leukemias

In fetal human red cells *i* antigen and f[illegible] hemoglobin (HbF) disappear during the postn[illegible] year In juvenile chronic myeloid leukemia [illegible] reappearance of HbF is common as it is in a lo[illegible] frequency in AML ALL erythroleukemia C[illegible] and CLL (25) It is not entirely clear whether [illegible] is revertance or a malignancy of HbF produc[illegible] stem cells

Oncogenic herpesviruses and small integra[illegible] DNA viruses induce enzymes for cellular D[illegible] synthesis and transform cells of other species [illegible]ceptionally this also happens in the species in wh[illegible] they are naturally replicating Defective rather t[illegible] infectious CMV particles induce cellular D[illegible] synthesis viral antigens are produced preferenti[illegible] in cells with no measurable DNA synthesis ([illegible] Furthermore the members of the herpesv[illegible] group induce Fc receptors in lytic infections of c[illegible] that usually do not express these proteins

EBV genomes are integrated in tumor cells [illegible] Burkitt lymphoma and nasopharyngeal carcino[illegible] (13) Virus antigens are readily detectable a[illegible] treatment of lymphoma cells with DNA analogu[illegible] Both specific cell mediated immunity antiviral a[illegible] bodies and heterophile antibodies directed a[illegible] variety of non viral proteins are induced by EBV [illegible] mononucleosis

There is some evidence that the heteroph[illegible] antibodies occurring in high titers of mononucleo[illegible] patients are due to expression of heteroph[illegible] antigens in hematopoietic tissue (12) Another [illegible]planation of heterophile antibodies in this disease [illegible] a preferential viral stimulation of a lymphoid [illegible] clone retaining ancient capacity to produce s[illegible] antibodies

Functional behavior of fetal antigens

The occurrence of fetal equivalents in tumors [illegible] stimulated several hypotheses about their fu[illegible]tions They concern alterations of *cellular grow[illegible]*

differentiation and *immune status* A few examples are given below indicating the mutual dependence of cell status on one hand and viral or fetal antigen synthesis on the other

Cell growth regulation Yolk sac tumors of rats can be induced following fetectomy and inoculation of an exogenous murine sarcoma virus (22) These tumors like human yolk sac and embryonal cancers produce AFP at high levels The induction of teratoid tumors producing fetal like AFP in animals has thus been related to virus inoculation and cellular transformation

The G_1 phase of the cell cycle is implicated by many authors as the cellular phase for maximal tumor antigen synthesis Viruses too are cell phase dependent for maximal virus release Moloney leukemia virus replicates optimally in dividing cells and G_1 seems to be the phase in which most virus is released from the cells (18)

Cellular differentiation Fetal and adult hemoglobins are the most prominent examples of gene products being expressed at varying times during development Viral infections too influence their expression Avian sarcoma virus induces the expression of fetal but not adult globin genes whole transformation defective mutants do not induce fetal genes (8)

Baluda (1) showed that development of chicken kidney tumors was dependent on the age of the chicken at the time of avian myeloblastosis viral infection A certain differentiation of the kidney with immature cells susceptible to virus transformation was therefore postulated

Stonehill and Bendich (20) have found several virus induced tumors which have the same fetal antigens as found in embryonal differentiation These antigens increase in concentration up to 19 days of embryonic life after which they decline except in skin

An antigen called F9 is present in morula embryonal cells and murine embryonal carcinoma cells as well (10) It is controlled by known genes at the T/t locus There is no known relation between viral infection and F9 expression but this system would perhaps provide means of studying viral influence upon regulation of differentiation at this early stage Recently Jaenisch et al (11) showed that an RNA virus when inoculated at the 4–8 cell embryonic stage integrated in the germ line The offspring was heterozygous for the leukemia inducing gene

Thus with some viruses new cellular functions are noted Such examples are reactivation of embryonal antigens or functions which otherwise are not expressed such as Fc receptors induced by herpesviruses interferon induced by many viruses heterophile antigens Tumor virus induced transplantation antigens and endogenous virus products also change the host cell but are mainly coded for by the integrated virus genome

Immunomodulation Pregnancy associated and embryonic antigens have recently been proposed to have immunoregulatory functions A suppressive effect on T cells has been ascribed to AFP HCG and pregnancy associated macroglobulin (PAM) Antibodies are generally not evoked to human fetal antigens during pregnancy or in tumor disease but may be so under exceptional circumstances or after an advertant break of tolerance

CONCLUSIONS

Different embryo antigens are probably recalled by different viruses otherwise cross reactivities between tumors induced by different viruses should be common (14) If the production of fetal antigens is a result of genome derepressions these changes could take place in diseases other than cancer Viral infections and congenital abnormalities are some of the other conditions in which we find fetal antigens

Available evidence suggests that oncogenic viruses of animals should become tools in investigating the relationship of fetal antigens to tumor transformation Fetal antigens in turn may become means to evaluate the role of virus influence for malignant transformation even of human cells

The group specific antigens of murine leukemia and sarcoma RNA viruses occur in a fashion similar to non viral fetal antigens These products are readily detectable during embryogenesis in low levels in the healthy adult and in increased amounts in certain tumors They provide assessment of cancer or recurrence risk As an excellent model in the animal system the association of a viral glycoprotein gp 52 to mammary tumors in mice and its good relationship in plasma to tumor status is of immediate interest (19)

DNA viruses are not known to be inherited via germ cells under natural conditions Some DNA viruses nevertheless have been associated with newly induced cellular information Also experi

mental tumors induced by chemical carcinogens have antigens in common with the fetus Mutagenic agents may therefore in addition to inducing new antigens change the genome so that cellular genes are expressed

It therefore seems important to characterize immunochemically and clinically all the products of human tumor cells which differ in occurrence or amount from normal cells in order to obtain tools with which to study the etiology of human tumors in vitro and in vivo

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REFERENCES

1 Baluda M Virology 32 428 1967
2 Benveniste R. E & Todaro G J Nature 261 101 1976
3 Berman L Int J Cancer 10 326 1972
4 Coggin J H Jr & Anderson N G In Embryonic and fetal antigens in cancer Proc of the second Conf Oak Ridge National Laboratory USA vol 2 p 91 1972
5 — Adv Cancer Res 19 105 1974
6 Gadler H & Wahren B Scand J Infect Dis 10 101 1978
7 Gold P Ann Rev Med 22 85 1971
8 Groudine M & Weintraub H Proc Natl Acad Sci 72 4464 1976
9 Hehlmann R Curr Top Microbiol Immunol 73 141 1976
10 Jacob F Immunol Rev 33 3 1977
11 Jaenisch R Berns A Dausman J & Cox V In Animal virology (ed D Baltimore A S Huang & C F Fox) p 283 Academic Press New York 1976
12 Kano K & Milgrom F Curr Top Microbiol Immunol 77 43 1977
13 Klein G In The herpesviruses (ed A S Kaplan) p 521 Academic Press New York 1973
14 Koprowski H Sawicki W & Koldovsky P J Natl Cancer Inst 46 1317 1971
15 Larouze B Blumberg B S London W T Lustbader E D Sankale M & Payet M J Natl Cancer Inst 58 1557, 1977
16 de Marchi J & Kaplan A Virology 82 93 1977
17 Maugh T H CEA Editorial Science 197 544 1977
18 Paskind M P Weinberg R A & Baltimore D Virology 67 242 1975
19 Ritzi E Martin D S Stolfi R L & Spiegelman S J Exp Med 145 999 1977
20 Stonehill E H & Bendich A Nature 228 370 1970
21 Strand M August J T & Jaenisch R Virology 76 886 1977
22 Vandeputte M Sobis H Billiau A van de Maele B & Lyten R Int J Cancer 11 536 1973
23 Wahren B Alpert E & Esposti P J Natl Cancer Inst 58 489 1977
24 Waldenstrom J G In Health control in detection of cancer Skandia Int Symposia p 118 Almqvist & Wiksell Stockholm 1976
25 Weatherall D J Clegg J B Wood W G Callender S T Sheridan B L & Pritchard J Nature 257 710 1975

Additional references to literature pertinent to this article can be obtained from the author

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Interferon Therapy in Multiple Myeloma

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ABSTRACT A woman with multiple myeloma relapsed after 6 years of satisfactory tumor control with melphalan therapy When progression then occurred, she was given exogenous human leukocyte interferon, 3×10^6 reference units twice daily i m as the sole therapy Side-effects of the interferon therapy consisted of fever reactions and thrombocytopenia One month after the initiation of interferon therapy there was 1) improvement of general health with less pain and tiredness, 2) reduction of the M component, IgG lambda, in the serum and 3) a reduced plasma cell concentration in the bone marrow After 5 months of interferon therapy tumor progression occurred despite continuous interferon treatment At the same time, the tumor cells were less sensitive to interferon in in vitro tests than prior to interferon therapy It is suggested that interferon therapy should be given as initial treatment to a few patients with multiple myeloma in a phase I trial

Key words interferon multiple myeloma
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High dose exogenous human leukocyte interferon administration to man has been possible without giving rise to considerable side effects (1 5 16 17 18) At the Karolinska Hospital such administration has been under study since 1969 and we have previously reported on the possible antineoplastic potency of such preparations (1 5 16 17 18)

We decided to study whether a case of myeloma would respond to interferon therapy since (*a*) myeloma cells have been found to be sensitive to interferon in tissue culture (6) and (*b*) it was considered possible to follow the interferon sensitivity of myeloma cells in the marrow of interferon treated patients Moreover multiple myeloma is a suitable disease for the study of drug effects since the state of the disease can be followed closely by 1) examining the bone marrow and 2) determining the extent of tumor specific protein production (2 20) The response of a melphalan resistant myeloma case to exogenous leukocyte interferon therapy is the subject of this report

MATERIALS AND METHODS

Interferon preparation

Interferon was prepared from human leukocyte cultures as described in detail previously (13) Partial purification was achieved to give a specific activity of about 10^6 U per mg of protein (5) The preparations employed contained approximately 10×10^6 reference units/ml

Interferon treatment

The interferon was injected i m The dose schedule is stated in the case report below As found in other patients receiving human leukocyte therapy the myeloma patient was subfebrile during the course of therapy (10) Antipyretics had to be given sometimes but the fever reaction never disturbed the patient extensively and discontinuation of the interferon therapy had never to be considered

Cytostatic regimen

Melphalan (Alkeran®) had been given prior to interferon therapy as stated in the case report below

Bone marrow examination

To evaluate plasma cell infiltration aspiration bone marrow biopsies were performed on the sternum on 8 occasions and on the posterior iliac spine on one All specimens were obtained utilizing the Kifa Franzen aspiration syringe and the Franzen bone marrow cannula Smears were made from the aspirate and routinely stained with May Grunwald Giemsa stain When sufficient material was available—in 7 out of 9 biopsies—marrow fragments were collected fixed in formalin and processed by routine histologic techniques (11) Bone marrow cellularity was

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Table I *Bone marrow findings before and during interferon therapy*

Date of sampling	Histological sections: Cellularity	Pattern of plasma cell infiltration: Diffuse	Nodular	Cytological smears: Pattern of plasma cell distribution	No of bone marrow cells counted	Plasma cells (%)
March 26 1969[a]	2+	1+	0	Diffuse	4 010	8 5
May 28 1969[b]	2+	1+	0	Diffuse	4 805	11 5
July 1 1970[b]	2+	1+	0	Diffuse	2 326	4 7
Aug 25 1975[b]	3+	2+	1+	Diffuse	1 595	34 5
Oct 7 1975				Diffuse	1 978	37 0
Oct 20 1975	5+	2+	2+	Diffuse	2 326	45 5
Dec 1 1975[c]				Diffuse and focal	725	8 5
Jan 20 1976[c]	3+	2+	2+	Diffuse and focal	1 859	60 4
May 12 1976	4+	3+	3+	Diffuse and focal	1 584	83 0

[a] At the time of diagnosis [b] during melphalan therapy [c] during interferon therapy

assessed in histological sections and expressed on a scale of 1+ to 5+ The pattern of plasma cell infiltration diffuse or nodular was noted and expressed on a scale of 0 to 3+ Differential counts of bone marrow were performed on the smears and the relative number of plasma cells was calculated as per cent of the number of nucleated marrow cells Depending upon the number of smears and the availability of areas where the cells were well preserved 725–4 800 cells were counted Cell counts were carried out without knowledge of the clinical background

Tests for interferon sensitivity in vitro

Preparation of cells Bone marrow aspirates were collected two occasions (Oct 22 1975 and May 3 1976) in ed tubes containing tissue culture medium RPMI 1640 supplemented with 10% calf serum and antibiotics) and transported to the laboratory within one hour Myeloma cells were then separated from erythrocytes and granulocytes by buoyant gradient centrifugation (4) The adherent cells were removed by 2 hours incubation of the separate cells in plastic Petri dishes The fraction of myeloma cells in mononuclear cell populations was determined on cytocentrifuge smears Total cell number was calculated by an electronic cell counter The viability was estimated by trypan blue exclusion

Oct 1975 The sensitivity of the patient s (M L) myeloma cells to interferon was compared in vitro with that of the two established human myeloma cell lines RPMI 8226 (12) and U 266 (14) as follows the growth in duplicate cultures (2×10^5 cells/2 ml of Ham s F 10 medium containing 10% fetal calf serum and 0 10 100 or 1 000 IU/ml of interferon) of the M L RPMI 8226 and U 266 myeloma cells was determined by counting the total and viable cell number after 7 days of incubation at 37°C in a 5% CO_2 in air atmosphere One ml of fresh medium containing interferon was replaced on day 5

May 1976 This time the effect of interferon on growth of the separated M L myeloma cells was studied by cell counting as described above and by ^3H thymidine incorporation as detailed (8 15)

Laboratory investigations

ESR Hb WBC differential counts platelet counts serum calcium serum creatinine and liver tests were all determined according to standard procedures employed at the hospital Electrophoresis was done regularly by the hospital laboratory according to standard procedures The sera were also then analyzed in parallel tests for content of tumor specific IgG by electroimmunoassay according to Grubb

CASE REPORT

Clinical history

The patient a woman born in 1910 had had a commotio cerebri in 1948 and had been suffering from lumbago for several years She had had iterated erysipelas infections for a couple of years before 1969 when she consulted a doctor because of increased tiredness and migrating arthralgia

Investigations at that time revealed ESR 42 mm/h Hb 118 g/l WBC $4\ 5\times10^9$/l with a normal differential count The platelet count was 195×10^9/l An M component type IgG lambda 30 g/l was found in her serum together with a lowered IgA concentration but normal IgM and albumin concentrations No light chains were found in the urine S calcium and S creatinine were normal An X ray of the skeleton was normal except for moderate spondylosis in the thoracic and lumbar part of the spine Bone marrow examination revealed a normal fat content and ordinary cell concentration However they contained an increased number of plasma cells (Table I) The diagnosis of myeloma was made

The patient was treated p o daily with 4 mg of melphalan for two months The dose was then diminished to 2 mg every second or third day according to the WBC which was not allowed to fall below 2×10^9/l From the initiation of melphalan therapy up to Aug 1975 there was no

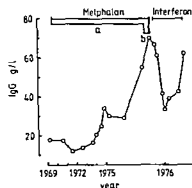


Fig 1 M-component concentration in serum at the time of diagnosis during melphalan therapy (*a*=4–6 mg weekly *b*=2 mg daily) and interferon therapy

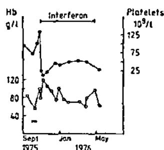


Fig 2 Hb concentration (O—O) platelet counts (●—●) and number of transfusions (↑=1 unit of erythrocytes) immediately before and during interferon therapy

real change in her disease state No clinical improvement was seen and she still suffered from pain and tiredness The ESR the M-component IgA IgM albumin and Hb concentrations remained essentially unchanged WBC was 2–4×10^9/l and platelets 70–100×10^9/l Bone marrow tests and repeated X rays of the skeleton did not reveal any signs of progression

In Aug 1975 however the M component rose to 56 g/l (Fig 1) the Hb concentration decreased to 78 g/l and the plasma cells constituted 34 5% of all nucleated cells in the bone marrow (Table I) The melphalan dose was then increased from 2 mg twice a week to 2 mg/day for 30 days Melphalan treatment was thereafter discontinued because of progression of the thrombocytopenia to 70×10^9/l and the anemia Hb 57 g/l The patient was transfused X ray examination revealed destructions of the skull but not of other parts of the skeleton The patient was in a poor clinical condition and suffered from bleedings from the nose gingiva and anus She had no petechial bleedings

In Oct 1975 when melphalan had been abandoned for 18 days it was decided not to reconsider this therapy because of assumed tumor resistance Interferon 3×10^6 U i m twice daily was given instead as single therapy from Oct 20 The patient had previously received 5 U of blood (Fig 2)

The body temperature increased by 2 0°C after the first interferon injection but normalized after an additional 48 hours without discontinuing the interferon treatment No antibiotics were given The patient was often subfebrile during the continued interferon therapy as experienced with other patients No infectious agent was ever detected

Three days after initiation of the interferon treatment platelet count fell markedly to 10–15×10^9/l (Fig 2) A slow increase was noted thereafter but platelets never rose above 70×10^9/l No bleeding tendency was noted and only minor erythrocyte transfusions were given later (Fig 2)

The M component in serum decreased the concentration in Dec 1975 being about half of the value measured in Oct (Fig 1) The patient felt subjectively better in Nov 1975 with less pain and tiredness but in Dec she again suffered from marked lumbago A new X ray showed no destructions at that time either She received local radiotherapy 4×150 rad to the lumbar spine with some positive effect Interferon therapy was continued In Feb 1976 an evident improvement occurred with less pain and tiredness

In March the patient developed heart failure which was easily cured by digitalization and diuretics

In April 1976 her condition very quickly deteriorated with reduced appetite weight loss and tiredness An X ray revealed destructions in the pelvis The M component had started to increase already in March but was still higher in April (Fig 1) The interferon therapy was discontinued on April 28 The patient died on May 8 1976

Autopsy revealed generalized myeloma with tumor infiltration of bone marrow spine and skull but myeloma cells were not detected in other organs There were also signs of bronchopneumonia and pulmonary edema

Besides determinations of Hb and M-component concentrations and platelet and WB counts some other laboratory tests were run continuously during the interferon therapy Liver tests (ALP ASAT ALAT bilirubin and GT) revealed normal and unchanged findings during therapy LD and haptoglobin determinations showed increased values on some occasions S-creatinine and S-calcium were normal Because of subfebrility several bacteriological (including tuberculosis) cultures from different organs were made all showing negative results

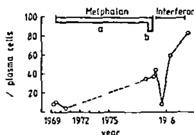


Fig 3 Plasma cells as % of total bone marrow cells at the time of diagnosis during melphalan therapy (*a*=4–6 mg weekly *b*=2 mg daily) and interferon therapy

Table II *Effect of interferon on the growth of human myeloma cells in vitro (Oct 1975)*

Interferon concentration (IU/ml)	Myeloma cells×10⁵/culture (7 days incubation) M L No of cells	M L Viability (%)	U 266 No of cells	U 266 Viability (%)	RPMI 8226 No of cells	RPMI 8226 Viability (%)
0	0 49	63	1 65	63	5 17	89
10	0 35	51	0 78	41	2 86	81
100	0 23	31	0 20	15	2 78	82
1 000	0 20	39	0 18	7	2 60	83

RESULTS

Bone marrow examination

The results of bone marrow examinations and cell counts are shown in Table I and illustrated in Fig 3 During the initial period of melphalan therapy although cellularity and fat content of the bone marrow were approximately normal diffuse plasma cell infiltration was present Plasma cell concentrations in the smears did not exceed 11 5% and the plasma cells showed a moderate variability in size and maturity During the final period of melphalan therapy (1975) bone marrow cellularity increased and diffuse infiltration became more prominent while nodular collections of plasma cells appeared The relative number of plasma cells in the smears had risen to 45 5%

[illegible] the start of interferon therapy a spec[illegible] drop down to 8 5% was observed in the per[illegible] of bone marrow plasma cells However despite continued interferon treatment bone marrow cellularity rose again and plasma cell infiltration progressed up to 83% In the last sternal puncture plasma cells still showed about the same variability in size and maturity as seen originally at the onset of the disease

In vitro test

Table II shows that the isolated bone marrow mononuclear cells of M L (91% of which were myeloma cells) were sensitive to interferon at all concentrations when tested in Oct 1975 A direct comparison of the interferon sensitivity with that of the established cells is not fully justified since the latter cells are exponentially growing (35% of the cells in the S-phase) while among the M L myeloma cells only a fraction seemed to be proliferating (2% S-phase cells) However the order of magnitude of the interferon sensitivity of the M L cells may be similar to that of the U 266 cells The RPMI 8266 cells were growth inhibited but the viability was unaffected

In May 1976 interferon at concentrations of 10 and 100 IU/ml did not affect the cell proliferation or viability (90% of the cell population were morphologically distinguishable myeloma cells) If any thing a slight growth stimulation was noted by increased cell number slightly higher viability and higher ^{3}H Tdr uptake after 7 days of incubation in the interferon treated cultures (Table III)

DISCUSSION

We selected this case of myeloma for treatment with human interferon therapy since the patient had tumor progression during ongoing melphalan therapy thrombocytopenia and since we previously had found that myeloma cells are sensitive to interferon in tissue culture (Einhorn and Strander unpublished results) Melphalan can cause late effects on various myeloma parameters and therefore the possibility has to be considered that the decrease in tumor mass after initiating interferon therapy could be due to late effects of melphalan given It is more likely however that the tumor decrease was due to

Table III *Effect of interferon on the growth of M L myeloma cells in vitro (May 1976)*

Interferon concentration (IU/ml)	Cell counts Total no of cells	Cell counts Viability (%)	^{3}H Tdr incorporation (cpm)
0	6×10⁵	53	3 715
10	4 6×10⁵	73	6 233
100	6 3×10⁵	65	13 099

the interferon therapy since some time (18 days) elapsed between melphalan and interferon treatments and since there was a change in the interferon sensitivity of the myeloma cells in tissue culture during the interferon treatment period when clinically regression was followed by progression

In connection with the clinical improvement a decrease was registered in the various parameters employed for the determination of the outcome of myeloma The number of bone marrow plasma cells and the amount of M component in the serum decreased and the Hb values stabilized In April 1976 the general condition of the patient deteriorated and at the same time the various laboratory tests revealed a progression

The in vitro results correlated well with the clinical observations Plasma cells decreased dramatically after initiation of interferon therapy a decrease that can hardly be explained by variability of the technique

It was evident from the interferon sensitivity tests in tissue culture that the cells tended to be more resistant to interferon after some time of therapy It is known that tumor cells may become resistant to interferon during long term cultivation in the presence of interferon in vitro (9) In this connection it is of interest that myeloma cells have been reported to be capable of producing interferon (7) a finding that may indicate ability to become resistant

We have previously observed a similar clinical result with initial improvement followed by progression during ongoing interferon therapy in a case of generalized Hodgkin s disease (3) Some interferon treated osteosarcoma patients developed metastases during ongoing interferon therapy (1 19)

The treatment of our patient was of simple nature—the injections were given by a district nurse and no dramatic side effects were reported It is therefore our intention to treat multiple myeloma patients with interferon as early as possible in the disease i e immediately after establishing the diagnosis and before any other treatment has been instituted Preliminary results on such patients are encouraging

ACKNOWLEDGEMENTS

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REFERENCES

1 Adamson U Aparisi T Brostrom L Å Cantell K Einhorn S Hall K Ingimarsson S Nilsonne U & Soderberg G Interferon treatment of human osteosarcoma Study week of the Pontificial Academy of Sciences Vatican City Oct 17–21 1977 "The Role of Non Specific Immunity in the Prevention and Treatment of Cancer In press 1977
2 Bergsagel D Plasma cell neoplasms In Cancer medicine (ed J F Holland and E Frei III) pp 1330–1358 Lea & Febiger Philadelphia 1973
3 Blomgren H Cantell K Johansson B Lagergren C Ringborg U & Strander H Interferon therapy in Hodgkin s disease—A case report Acta Med Scand 199 527 1975
4 Boyum A Separation of leukocytes from blood and bone marrow Scand J Clin Lab Invest (Suppl) 97 51 1968
5 Cantell K & Strander H Human leukocyte interferon for clinical use In Blood leukocytes Function and use in therapy Symposium Uppsala Oct 10–13 (ed C F Hogman K Lindahl Kiessling and H Wigzell) pp 73–75 1977
6 Einhorn S & Strander H Interferon therapy for neoplastic diseases in man In Production of human interferon and investigations of its clinical use A Symposium Workshop W Alton Jones Cell Science Center Lake Placid USA May 19–20 1977
7 Epstein L B & Salmon S E Production of interferon by malignant plasma cells from patients with multiple myeloma. J Immunol 112 1131 1974
8 Ghetie V Nilsson K & Sjoquist J Density gradient centrifugation of lymphoid cells adhered to protein A containing staphylococci Proc Natl Acad Sci (USA) 71 4831 1974
9 Gresser I Antitumor effects of interferon In Cancer—a comprehensive treatise vol 5 Chemotherapy pp 521–571 Plenum Press New York 1977
10 Ingimarsson S Cantell K & Strander H Symptomatic side-effects in patients receiving long term therapy with human leukocyte interferon J Infect Dis In press 1978
11 Larsson L -G & Franzen S Sternal puncture in sarcoidosis Acta Radiol 37 59 1952
12 Matsuoka I Takahashi M Yagi E Moore G F & Pressman D Production of free light chains of immunoglobulin by a haematopoietic cell line derived from a patient with multiple myeloma Proc Soc Exp Biol (NY) 125 1 46 1967
13 Mogensen K E & Cantell K Production and preparation of human leukocyte interferon Pharmacol Ther C 1 369 1977
14 Nilsson K Bennich H Johansson S G O & Ponten J Established immunoglobulin producing myeloma (IgE) and lymphoblastoid (IgG) cell lines from an IgE myeloma patient Clin Exp Immunol 7 477 1970
15 Nilsson K Killander D Killander J & Mellstedt H Short term tissue culture of two non-secretory human myelomas Scand J Immunol 5 819 1976
16 Strander H Interferons Anti-neoplastic drugs? Blut 35 277 1977

17 Strander H & Cantell, K Studies on antiviral and antitumor effects on human leukocyte interferon in vitro and in vivo In The production and use of interferon for the treatment and prevention of human virus infections Proc of a Tissue Culture Association Workshop Tissue Culture Assoc Monograph no 3 pp 49–56 Rockville Maryland 1974
18 Strander H Cantell K Carlstrom G & Jakobsson P Clinical and laboratory investigations on man Systematic administration of potent interferon to man J Natl Cancer Inst 51 733 1973
19 Strander H Cantell K Ingimarsson S Jakobsson P Å Nilsonne U & Soderberg G Interferon treatment of osteogenic sarcoma—a clinical trial Conference on Modulation of host immune resistance in the prevention or treatment of induced neoplasias Dec 9–11 1974 Fogarty Int Center Proceedings no 28 pp 377–381 US Government Printing Office Washington DC In press 1977
20 Waldenstrom J Diagnosis and treatment of multiple myeloma Grune & Stratton New York 1970

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Metabolic Studies and Glucagon Gel Filtration Pattern before and after Surgery in a Case of Glucagonoma Syndrome

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ABSTRACT A case of glucagonoma syndrome with necrolytic migratory erythema, glossitis, anemia, hyperglucagonemia and a malignant, pancreatic A cell tumour in a 68-year old male is described Gel filtration of the highly elevated circulating glucagon immunoreactivity (2200 pg/ml) demonstrated 60% pancreatic glucagon and 30% "proglucagon" Metabolic studies before operation demonstrated suppression of the total plasma glucagon concentration on oral glucose tolerance test, unchanged total plasma glucagon concentration during intravenous glucose tolerance test and insulin induced hypoglycemia Administration of arginine was followed by a rise in both the pancreatic glucagon and the 'proglucagon", whereas alanine increased only the pancreatic glucagon The plasma somatostatin level was immeasurable preoperatively Somatostatin infusion completely suppressed the release of the pancreatic glucagon but did not significantly affect the 'proglucagon ' After removal of the tumour the skin lesions disappeared and the total plasma glucagon values fell to normal levels (120 pg/ml) Also other abnormal laboratory findings returned to normal including the preoperatively observed renal glucosuria

Key words glucagonoma syndrome pancreatic A cell tumour glucagon proglucagon big plasma glucagon glicentin metabolic studies

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The glucagonoma syndrome is an established clinical entity (14 20) with hyperglucagonemia pancreatic A-cell tumour necrolytic migratory erythema (4) usually decreased glucose tolerance weight loss and anemia Since there are cases reported with immunological hyperglucagonemia without elevation of the pancreatic hormone (5) the different immunoreactive forms of glucagon have to be identified by gel filtration of the plasma (5 8 16 31) in addition to determination of total glucagon (9)

A patient with a malignant glucagonoma in whom extensive studies were performed both pre per and postoperatively is presented here The distribution of the molecular size of the circulating glucagon was estimated The results of the metabolic studies performed are compared with those from previous reports

CASE REPORT

The patient is a male born in 1909 His mother and one sister suffered from diabetes mellitus of maturity onset type From 1958 to 1976 he noticed recurrent multiple skin eruptions localized particularly to the lower extremities and groins During 1965–77 he lost 16 kg in weight He experienced periods of diarrhoea gastric pain and fever

In 1976 there was progress of the skin symptoms and the patient was referred to the Department of Dermatology Symmetrically on the forelegs and on the backs of the feet there were multiple collapsed whitish blisters on a red edematous ground (Fig 1) Erythematous erosions with scaling were seen in the genital and perianal regions (Fig 2) In the face there was an erythema with fine scaling In connection with the progress of the dermatitis in 1976 a transient glucosuria was registered Intermittent glucosuria could however be traced back to 1963

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Abbreviations GLI = glucagon like immunoreactivity gut GLI = gastrointestinal GLI enteroglucagon

Table I. *Laboratory investigations*

B = blood, P = plasma, S = serum, ERY = erythrocyte, U = urine, tU = time period for collected urine. Somatostatin (3) was analyzed by Dr G. Lundqvist, Uppsala. Analyses of adenosine and guanosine cyclic 3 5 phosphate (C-AMP and C-GMP) were performed by Dr J. Malmquist, Malmö.

	Reference values	Patient's values: Pre operative	Post operative
B-hemoglobin (g/l)	1[illegible]1 163	109	1[illegible]1
ERY MCV (fl)	8[illegible] 10	97	93
ERY MCHC (g/l)	3[illegible]0–360	311	310
Erythrocyte sedimentation rate (mm/h)	<30	23	6
B-glucose (fasting) (mmol/l)	3 3–5 6	4 4	4 7
U-glucose (mmol/l)	<1	9–27	1 [illegible]
S-iron (μmol/l)	13–35	10 5	7 0
S-total iron binding capacity (μmol/l)	45–7[illegible]	41	54
S-calcium (mmol/l)	2 [illegible] [illegible] 6	[illegible]	[illegible] 4
tU-calcium (mmol/24 h)	[illegible] 5–8 0	[illegible] 3	3 6
S-albumin (g/l)	40–5[illegible]	31	35
S-cholesterol (fasting) (mmol/l)	3 6–8.3	3 4	5 0
P triglycerides (fasting) (mmol/l)	0 4–[illegible]	1 0	1.5
P-glucagon (total) (pg/ml)	50–250	[illegible] 000	1 0
P-somatostatin (pg/ml)	25–105	<10	79
P renin activity (supine) (μg/l 3h)	0 5–[illegible] 0	3 7	1 3
P renin activity (standing) (μg/l 3h)	1 0–4 0	23 9	3 6
P-C AMP (nmol/l)	[illegible] 10	7 0	7 7
tU-C AMP (nmol/mmol creat.)	150–600	488	361
P-C-GMP (nmol/l)	[illegible]–6	16 0	3 [illegible]
tU-C-GMP (nmol/mmol creat.)	0–50	89 8	45 4

On suspicion of a glucagonoma syndrome a punch biopsy of a skin lesion was performed. It demonstrated morphological features compatible with this syndrome. Plasma values of total glucagon measured by radioimmunoassay were highly elevated. Plasma amino acids were decreased as reported in other cases (20). Glucosuria was present but repeated fasting blood glucose values and an oral glucose tolerance test were normal. There was a normocytic slightly hypochromic anemia. An angiography revealed a highly vascularized tumour in the pancreatic tail.

The patient was admitted to the Department of Endocrinology for further evaluation in May 1977. He complained of a sore tongue, anorexia, fatigue and weight loss apart from the skin lesions. On physical examination he appeared thin and weak. He weighed 61 kg and measured 17[illegible] cm. The tongue was bright red with a complete papillary atrophy (Fig. 3). There were extensive skin lesions on the lower extremities. In the face there was an erythema with fine scaling. Supine blood pressure was 105/60 mmHg and an auricular fibrillation was found. It returned to sinus rhythm after 7 days on digoxin.

In June 1977 a laparotomy was performed. A tumour 4.5×6×5 cm was found in the distal part of the pancreas. The tumour was diffusely separated from the surrounding tissue but there were no signs of distant tumour spread. A catheter was introduced in the splenic vein and blood samples for glucagon assay were drawn at different distances from the tumour and simultaneously from a brachial artery. A resection of the pancreatic tail was performed.

The patient recuperated rapidly after the operation and the skin lesions healed within two weeks. On rehospitalization two months later he was in good condition, had gained 3 kg in weight and showed no signs of glossitis. One year after operation he had no recurrent hyperglucagonemia.

METHODS

Laboratory investigations

Laboratory tests were performed using routine methods except when indicated. Blood samples were drawn at 8.00 a.m. or as stated. Urine samples were collected during given periods. The metabolic studies were performed after an overnight fast.

Glucagon determination. Glucagon was determined by radioimmunoassay using highly purified porcine glucagon (Novo, Copenhagen) as standard and for iodination (34). Two pancreatic-type glucagon-measuring antisera, ET and 30K, were used (3?). The assay conditions were essentially those recommended by Falona and Unger (9) though normal sheep serum was omitted from the assay buffer. Antiserum-bound glucagon was separated from free glucagon by addition of plasma-coated charcoal. The detection limit of the assay was 10 pg glucagon/ml. The intra assay coefficient of variation at the 100–200 pg/ml levels was approximately 8%. Blood for the glucagon assay was treated as described elsewhere (3).

Gel chromatography of glucagon-containing plasma. To study the possible heterogeneity in molecular size of the immunoreactive forms of glucagon, plasma was sub-

jected to gel filtration A Biogel P 10 column (1 0×120 cm) was used for the studies with plasma from peripheral venous blood Another Biogel P 10 column (1 6×90 cm) was used for the plasma obtained from blood from the tumour-draining vessels and from a forearm artery during the operation The columns were kept at 22°C and eluted with assay buffer 0 2 mol/l glycine adjusted to pH 8 8 with sodium hydroxide containing human serum albumin 2 5 g/l (electrophoretically pure from Behringwerke Marburg Germany) Merthiolate® 0 1 mmol/l and Trasylol® 500×10³ KIU/l Samples of 2 ml plasma were applied Fractions containing 2 0 and 1 9 ml were collected at a flow rate of 15 ml/h and determined for pancreatic type glucagon immunoreactivity using antisera E7 and 30K Calibration of the columns was performed with ^{131}I albumin ^{125}I insulin and ^{125}I glucagon In addition the elution volume of the gastrointestinal glucagon like immunoreactivity (gut GLI) was determined by measuring the GLI content of the eluted fractions with antiserum 78J (22)

Metabolic studies

Oral glucose tolerance test (OGTT) and intravenous glucose tolerance test (IVGTT) were performed with 50 g of glucose at 10 p m on the day before the test 54 g glucose (30 g/m² body surface) were given at the OGTT and 43 g (25 g/m² body surface) at the IVGTT The same total dosages were used at the postoperative tests

Insulin induced hypoglycemia Insulin 0 1 IU/kg b wt (Actrapid® Novo Copenhagen) was given intravenously

Glucagon stimulation with alanine An infusion with 0 15 mol/l NaCl was started at a rate of 2 ml/min Thirty minutes later *l* alanine dissolved in sterile distilled water as a 10% (w/v) solution (adjusted to pH 7 4 by addition of sodium carbonate) was given in a total dose of 9 15 g during 2–4 min

Glucagon stimulation with arginine Arginine chloride dissolved in 0 15 mol/l NaCl was infused as a 10% (w/v) solution 13 mg/kg b wt /min during 30 min giving a total dose of 24 g of arginine

Nicotinic acid infusion Nicotinic acid dissolved in 0 15 mol/l NaCl was given initially as a 3 min bolus injection of 100 mg i v followed by an infusion of 1 000 mg for 57 min and thereafter 875 mg at a rate of 310 mg/hour

Glucagon suppression with somatostatin An infusion of cyclic somatostatin (Kabi Stockholm supplied by Dr S Efendic Stockholm) 10 µg/min was given during 90 min The somatostatin preparation was dissolved in 0 15 mol/l NaCl containing 5 g albumin/l

Histological and histochemical methods

Specimens from the pancreatic tumour were either frozen to the temperature of liquid nitrogen and sectioned in a cryostat or fixed in 10% formalin or Bouin s fluid embedded in paraffin and sectioned Specimens for electron microscopy were fixed in 2 5% glutaraldehyde postfixed in osmic acid and embedded in Vestopal W The tissue sections were stained with hematoxylin eosin and special stains were used for demonstration of normal islet A B and D cells The indirect immunohistochemical method (10) was used for demonstration of glucagon glicentin and insulin cells The glicentin antiserum R-64 (23) was applied in the laboratory of Dr L Orci Geneva Inhibition tests with glucagon and glicentin were performed with both glucagon and glicentin antisera Immunofluorescence of FITC labelled second antibodies was examined in a Leitz Orthoplan fluorescence microscope equipped with an epi illumination system Electron micrographs were taken with Philips EM 300 electron microscope

RESULTS

Laboratory data are listed together with the respective reference values in Table I The following blood analyses were normal before and after operation leucocytes platelets reticulocytes sodium potassium chloride phosphorus zinc magnesium creatinine peripheral thyroid hormones luteinizing and follicle stimulating hormones prolactin calcitonin vasoactive intestinal polypeptide (analysis Dr J Fahrenkrug Copenhagen) secretin (Dr Fahrenkrug) gut GLI carcinoembyronic antigen human chorionic gonadotrophin liver function enzyme tests The following urinary analyses were normal before and after operation aldosterone epinephrine norepinephrine 17 hydroxycorticosteroids 17 ketosteroids sodium potassium magnesium and zinc The P amino acids (analysis Dr P Fernlund Malmo) were generally decreased before operation and normalized after operation

The results from the metabolic studies are shown in Table II and the peroperative plasma glucagon determinations from the tumour draining vessels in Table III

Heterogeneity in molecular size of plasma glucagon immunoreactivity The profile on glucagon determination of the gel filtered plasma from peripheral venous blood is shown in Fig 4 upper panel Most of the immunoreactive glucagon eluted as the pancreatic glucagon marker A second peak of immunoreactivity considered to be proglucagon (18) was found between the insulin and the albumin marker A third peak of immunoreactivity was found in the void volume The shoulder in the ascending part of the second peak coincided with the gut GLI elution The lower panel in Fig 4 shows the chromatograms from tumour draining blood and arterial blood

Plasma samples during stimulation tests with arginine and alanine and the suppression test with somatostatin were also subjected to gel chromatography Samples taken before the test were compared with those taken when maximum response was found in the serial glucagon det

Table II *Results from metabolic studies performed before (Pre) and after (Post) operation*

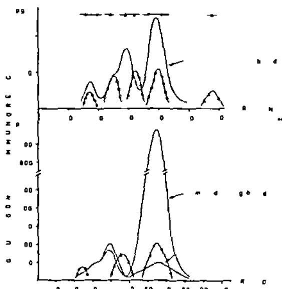
Time (min)	B-glucose (mmol/l) (26)		P-insulin (mIU/l) (12)		P-glucagon (pg/ml)		P-GH (μg/l) (40)		B-glucose (mmol/l)		P-insulin (mIU/l)		P glucagon (pg/ml)		P-GH (μg/l)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Oral glucose tolerance test									*Insulin-induced hypoglycemia*							
−10	4.1	4.1							4.2	4.1			2 000	130	8	8
±0	4.1	4.3	8	<3	1 900	120	2	3	4.0	4.1			1 900	140	8	8
+15									2.8	2.9						
+30	7.9	9.0	110	65	2 000	120	3	3	2.2	1.6			2 000	135	1	4
+45	8.8	9.3							1.9	1.5			1 700	145	4	4
+60	9.4	10.3	160	100	1 800	105	1	3	1.8	2.0			2 000	155	5	9
+90	7.0	7.7							2.8	3.1						
+120	4.9	5.3	100	44	1 400	110	1	3	3.6	3.6			2 400	130	14	34
+150	3.5	3.8														
+180	3.6	3.3	47	5	1 500	105	7	13								
Intravenous glucose tolerance test									*Suppression with somatostatin*							
−10	4.1	4.2							4.3		18		2 000		7	
±0	4.1	4.3	9	<3	1 900	125	4	2	4.1		15		1 900		5	
+5			120	84	1 900	120	5	3	3.4		10		1 300		5	
+8		17.4	100	72	1 700	120	4	3								
+10	12.2								3.5		7		1 400		5	
+15	10.9	13.6														
+20	12.4	13.0	140	43	1 900	120	3	3	3.6		4		900		4	
+30	11.3	11.7	150	40	2 000	95	3	3	3.2		3		900		3	
+40	9.7	10.4	150	41	1 800	90	2	2	2.9		5		700		2	
+50	8.7	9.3	140	44	1 900	90	1	2								
+60	7.4	8.2	140	43	1 800	120	7	3	2.6		3		700		1	
+70	6.5	7.6														
+90									2.6		<3		700		2	
Stimulation test with arginine									*Stimulation test with alanine*							
−30									3.8	4.3	10	7	2 100	125	7	4
−20		4.7		<3	4 000	115		4								
−10									3.8	4.3	10	<3	2 200	120	5	4
±0		4.7		<3	3 900	110		4	3.6	4.2	11	4	2 200	130	5	4
+10		4.8		8	17 200	135		4	4.3	4.2	52	15	7 700	150	5	3
		4.8		10	10 400	150		4	4.5	4.2	54	9	3 900	140	7	3
		4.9		8	8 500	150		4	4.2	4.2	46	9	2 800	130	6	3
*		4.8		7	5 500	155		11	4.2	4.1	32	7	2 500	135	6	3
+50		4.6		<3		135		24	4.1	4.3	24	6	2 000	140	8	3
+60		4.6		<3		130		27	3.9	4.3	16	5	2 000	160	8	5
+90									3.9	4.3	11	5	1 800	140	8	12
+120									3.7	4.5	9	6	2 100	140	5	7
									P-glycerol* (μmol/l) (19)		P FFA* (mmol/l) (38)					
Nicotinic acid infusion																
−30	4.0		8		2 200		13		37		0.2[illegible]					
±0	4.0		<3		2 200		11		44		0.24					
+30	4.4		<3		2 800		15		24		0.12					
+60	4.4		<3		2 200		12		20		0.06					
+90	3.9		<3		2 500		11		10		0.04					
+120	3.8		<3		2 400		9		14		0.04					
+150	3.9		<3		1 700		21		17		0.06					
+180	3.6		<3		1 900		35		17		0.02					
+210	3.8		<3		1 600		50		17		0.10					
+240	4.3		<3		3 800		46		24		0.12					

* Analyses performed by Dr G Fex Malmö

Table III *Deter t o t f t t l gl c go t o e ct t pl s fr t o r dra g bloo l obt ed d r g s rger*

B ood was drawn w th a cathe er nserted 1 cm n o the po tal e n (I) The ca heter was then forwarded n o the l enal c e n towards the tumour n the panc ea c ta l Blood was drawn at pos on II wh ch s 5 cm from pos on I and from pos tions III and IV each at a further d s an e of 5 cm Blood was also drawn from a forearm artery s mu aneously w h samples I II III and IV The lat er values a e given n paren he es

Pos on	An serum F 7 (pg/ml)	An serum 30 K (pg/ml)
I	4 800 (5 000)	5 000 (5 000)
II	4 700 (4 800)	5 000 (4 000)
III	>12 000 ( )	>12 000 ( )
IV	>12 000 (4 000)	>12 000 (4 000)



F g 4 Gel f tra on pa erns of plasma glucagon m munoreac v ty from a patient w h a glucagon produc ng tumour *Upper panel* Gel ch oma ography on B ogel P 10 (1 0×1 0 cm column) of peripheral enous blood 2 mon hs befo e opera on The l nes w th the symbols at the top of the f gure describe markers used for co umn cal bration Glucagon was assayed w h an sera E7 and 30K bo h show ng an den cal prof le *Lc er pa el* Gel chro ma ography on B ogel P 10 (1 6×90 cm column) of u mou d a n ng and arterial blood obta ned during opera on *Glucagon as ay w h antiserum E 7*

nat ons of the test (Table II) In all tests changes n mmunoreactive glucagon concentrations were found in the elut on fractions correspond ng to the pancreatic glucagon marker Sign f cant ncreases were recorded 20 m n after arg n ne st mulat on in both the pancreatic glucagon and the proglucagon peak Alanine increased only the glucagon peak After 60 min infusion of somatostat n no pancreat c glucagon could be detected but the other fract ons rema ned unaltered

T ss e f nd g s The pa icreatic tumour measured approx mately 4 5×5×6 cm and we ghed about 100 g It was partly well c rcumscribed and encap sulated but n some areas there was gross nf ltra t on nto the adjacent panc eat c t ssue wh ch showed a marked fat nvolut on

The tumour t ssue was composed of nests and trabecules of polyhedric cells wh ch were usually somewhat larger than normal slet cells The clusters of cells were often broken up nto a ribbon l ke pattern composed of s ngle rows of cyl ndr c cells ly ng n pal sades The degree of cytolog cal atyp a varied Less well d fferent ated areas showed marked cellular and nuclear pleomorphism and sev eral m tot c f gures The tumour had nfiltrated nto the surround ng pancreat c and ad pose t ssue There was per neural and vascular invas on fea tures wh ch were judged as ev dence of mal gnancy

The majority of the tumour cells showed stain ng characterist cs correspond ng to normal pancreat c glucagon cells (A cells) e g they were argyroph l w th Grimel us method (11) but d d not stain with s lver us ng he techn que of Hellerstrom and Hellman (13) nor d d they sta n w th aldehyde fuchs n (17)

Immunofluorescence demonstrated glucagon gl centin and n ul n cells Glucagon was also pres ent in the predom nant ant glicent n reacting cells S ngle or clustered tumour cells d splayed glucagon immunoreact v ty of vary ng intens ty In add t on occas onal cells w th n the tumour conta ned insul n mmuno eact ve material (B cells)

Ultrastructural observat ons revealed intracellu lar dense secretory granules (F g 5) They resem bled the A cell granules described in other gluca gonomas (7 24) The r number varied cons de a bly from one cell to another and also n d fferent parts of the tumour Cells completely devo d of granules seemed to be less well d fferent ated

DISCUSSION

Gl g gel f l a o pa er

Th s case of glucagonoma syndrome had very ex cess ve levels of glucagon mmunoreactiv t

Table II *Results from metabolic studies performed before (Pre) and after (Post) operation*

Time (min)	B glucose (mmol/l) (26)		P insulin (mIU/l) (12)		P glucagon (pg/ml)		P GH (µg/l (40)		B glucose (mmol/l)		P insulin (mIU/l)		P glucagon (pg/ml)		P-GH (µg/l)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Oral glucose tolerance test									*Insulin induced hypoglycemia*							
−10	4 1	4 1							4 2	4 1			2 000	130	8	8
±0	4 1	4 3	8	<3	1 900	120	2	3	4 0	4 1			1 900	130	8	8
+15									2 8	2 9						
+30	7 9	9 0	110	65	2 000	120	3	3	2 2	1 6			2 000	135	1	4
+45	*8 8*	*9 3*							*1 9*	*1 5*			*1 700*	*145*	*4*	*4*
+60	9 4	10 3	160	100	1 800	105	1	3	1 8	2 0			2 000	155	5	9
+90	7 0	7 7							2 8	3 1						
+120	4 9	5 3	100	44	1 400	110	1	3	3 6	3 6			2 400	130	14	34
+150	3 5	3 8														
+180	3 6	3 3	47	5	1 500	105	7	13								
Intravenous glucose tolerance test									*Suppression with somatostatin*							
−10	4 1	4 2							4 3		18		2 000		7	
±0	4 1	4 3	9	<3	1 900	125	4	2	4 1		15		1 900		5	
+5			120	84	1 900	120	5	3	3 4		10		1 300		5	
+8		17 4	100	72	1 700	120	4	3								
+10	12 2								3 5		7		1 400		5	
+15	10 9	13 6														
+20	12 4	13 0	130	43	1 900	120	3	3	3 6		4		900		4	
+30	11 3	11 7	130	40	2 000	95	3	3	3 2		3		900		3	
+40	9 7	10 4	130	41	1 800	90	2	2	2 9		5		700		2	
+50	8 7	9 3	140	44	1 900	90	1	2								
+60	7 4	8 2	140	43	1 800	120	7	3	2 6		3		700		1	
+70	6 5	7 6														
+90									2 6		<3		700		2	
Stimulation test with arginine									*Stimulation test with alanine*							
−30									3 8	4 3	10	7	2 100	125	7	4
−20		4 7		<3	4 000	115		4								
−10									3 8	4 3	10	<3	2 200	120	5	4
±0		4 7		<3	3 900	110		4	3 6	4 2	11	4	2 200	130	5	4
+10		4 8		8	12 200	135		4	4 3	4 2	52	15	7 700	130	5	3
+20		4 8		10	10 400	150		4	4 5	4 2	54	9	3 900	130	7	3
+30		4 9		8	8 500	150		4	4 2	4 2	46	9	2 600	130	6	3
+40		4 8		7	5 500	155		11	4 2	4 1	32	7	2 500	135	6	3
+50		4 6		<3		135		24	4 1	4 3	24	6	2 000	140	8	3
+60		4 6		<3		130		27	3 9	4 3	16	5	2 000	160	8	5
+90									3 9	4 3	11	5	1 800	140	8	12
+120									*3 7*	*4 5*	*9*	*6*	*2 100*	*140*	5	7
Nicotinic acid infusion									P-glycerol (µmol/l) (19)		P FFA (mmol/l) (38)					
−30	4 0		8		2 200		13		37		0 22					
±0	4 0		<3		2 200		11		44		0 24					
+30	4 4		<3		2 800		15		24		0 12					
+60	4 4		<3		2 200		12		20		0 06					
+90	3 9		<3		2 500		11		10		0 04					
+120	3 8		<3		2 400		9		14		0 04					
+150	3 9		<3		1 700		21		17		0 06					
+180	3 6		<3		1 900		35		17		0 02					
+210	3 8		<3		1 600		50		17		0 10					
+240	4 3		<3		3 800		46		24		0 12					

[a] Analyses performed by Dr G Fex Malmo



Fg 1 Necrolyt c m gratory erythema appearing symmetr cally on the ankles and c 1 tl e l 1cks of the feet The collapsed wh t sh bl sters are partly shed



*F*_{k} *2* Erytl ma ous eros ons th scal n and cru s n tl e sc o al and ngu nal reg n



F 3 Th ngue br ght r d v th pap llary a rop

amino acids in the epidermis resulting in epidermal lesions

Metabolic studies

It has been speculated that the glucose sensing function of the A cell resides not in the A cell but in the B-cell which transmits the signal required for an appropriate A cell response to hypoglycemia (39) When A cells are separated from B cells their glucose sensing capacity would thus be lost In accordance with this theory glucagon release by glucagon secreting islet cell tumours (6 28 37) is reported to be unaffected by glucose Danforth et al (8) found on the contrary a decrease in glucagon following glucose intravenously Valverde et al (41) found a slight suppression of plasma glucagon during an OGTT in a glucagonoma We found a significant decrease in glucagon following oral but not intravenous glucose This finding may be coincidental but could also indicate that glucagon release is regulated by other factors beside the glucose concentration

Alanine and arginine are both well known stimulants of insulin and glucagon secretion but there are contradictory results concerning the influence of arginine on glucagon secretion by glucagonomas and glucagon secreting islet tumours (6 21 28 36 37) In the present case glucagon was greatly increased when stimulated with these amino acids Gel chromatography of plasma revealed that the increase consisted of pancreatic glucagon Arginine also stimulated the release of proglucagon Similar findings were reported by Weir et al (42) A sluggish and much less pronounced increase in glucagon following alanine was found in our patient after operation In spite of the high peak of glucagon following alanine stimulation before operation blood glucose increased only from 3 6 to 4 5 mmol/l This emphasizes a relatively small influence of even large quantities of glucagon on the carbohydrate metabolism in this case

Following nicotinic acid there was an expected decrease in free fatty acids and an increase in growth hormone whereas glucagon demonstrated a paradoxical decrease followed after 4 hours by a rapid increase There are several mechanisms (2 30) by which a decrease in plasma free fatty acids could possibly affect glucagon secretion However an interpretation of our data is difficult since our patient became nauseated and vomited during the latter part of the infusion

An interesting observation not reported before is that the somatostatin concentration in peripheral blood before operation was immeasurable but returned to normal two months after operation Normally somatostatin causes a decrease in glucagon insulin growth hormone and blood glucose In a case of glucagonoma described by Mortimer et al (25) somatostatin infusion decreased glucagon and glucose while insulin level did not change However our patient reacted with a decrease in glucagon and blood glucose as well as insulin and growth hormone during somatostatin infusion The pancreatic glucagon disappeared completely as demonstrated by gel chromatography The other immunoreactive fractions of higher molecular weight did not change significantly in concentration This is noteworthy since it suggests that proglucagon is not suppressed within 60 min of the somatostatin infusion

In a case report of a glucagonoma Danforth et al (8) found very high values of plasma vasoactive intestinal polypeptide and serum carcinoembryonic antigen Both were normal in our patient

Cardiac effects

The finding of an auricular fibrillation may be coincidental However it may also be directly related since glucagon—apart from the well known positive inotropic effects—also has a positive chronotropic effect (27)

Glucosuria

Before operation our patient had a constant glucosuria of 9–27 mmol/l in spite of normal blood glucose levels This glucosuria probably of renal origin decreased after operation to 1–2 mmol/l This could reflect an influence of glucagon on tubular reabsorption of glucose but this problem was not further studied Previously van Itallie et al (15) found no effect on glucose tubular maximum in two subjects In dogs there are reports of a higher glucose excretion rate from glucagon infused kidneys (29) and glucagon causing a decreased ratio between tubular maximum and glomerular filtration rate (35)

ACKNOWLEDGEMENTS

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REFERENCES

1 Alford F P Bloom S R & Nabarro J D N Diabetologia 13 1 1977
2 Andrews S S Lopez S A & Blackard W G Metabolism 24 35 1975
3 Arimura A Lundqvist G Rothman J Chang R Fernandez Durango R Elde R Coy D H Meyers C & Schally A V Metabolism (Suppl) 1 1139 1978
4 Binnick A N Spencer S K Dennison W L & Horton E S Arch Dermatol 113 749 1977
5 Boden G & Owen O E N Engl J Med 296 534 1977
6 Boden G Owen O E & Quickel K E Diabetes (Suppl) 1 341 (Abstract) 1976
7 Capella C Solcia E Frigerio R Usselini L & Fontana P Virchows Arch (Pathol Anat) 373 327 1977
8 Danforth D N Jr Triche T Doppman J L Beazely R M Perrino P V & Recant L N Engl J Med 295 242 1976
9 Faloona G R & Unger R H In Methods of hormone radioimmunoassay (ed B M Jaffe & H R Behrman) p 317 Academic Press New York 1974
10 Goldman M Fluorescent antibody methods Academic Press New York 1968
11 Grimelius L Acta Soc Med Ups 73 243 1968
12 Heding L G In Labelled proteins in tracer studies (ed L Donato) p 345 Euratom Bruxelles 1966
13 Hellerstrom C & Hellman B Acta Endocrinol (Kbh) 35 518 1960
14 Holst J J Ugeskr Laeger 135 2627 1975
15 van Itallie T B Felber J P Hoet J & Renold A E Diabetes 8 94 1959
16 Jaspan J B & Rubenstein A H Diabetes 26 887 1977
17 Jennings B M J Histochem Cytochem 13 328 1965
18 Kuku S F Zeidler A Emmanouel D S Katz A I & Rubenstein A H J Clin Endocrinol Metab 42 173 1976
19 Laurell S & Tibbling G Clin Chim Acta 13 317 1966
20 Mallinson C N Bloom S R Warin A P Salmon P R & Cox B Lancet 2 1 1974
21 Mallinson C N Cox B & Bloom S R Gut 15 340 (Abstract) 1974
22 Marco J Hedo J A Villanueva M L Calle C Corujedo A & Segovia I M Diabetologia 13 131 1977
23 Moody A J Frandsen E K Jacobsen H Sundby F & Orci L Metabolism (Suppl) 1 1336 1976
24 McGavran H M Unger R H Recant L Polk H C Kilo C & Levin M E N Engl J Med 274 1408 1966
25 Mortimer C H Carr D Lind T Bloom S R Mallinson C N Schally A V Tunbridge W M Yeomans L Coy D H Kastin A Besser G M & Hall R Lancet 1 697 1974
26 Neely W E Clin Chem 18 509 1972
27 Parmley W W Glick G & Sonnenblick E H N Engl J Med 279 12 1968
28 Pek S Fajans S S Floyd J C Jr & Knopf R F In Diabetes (ed J W Malaisse & J Pirart) p 207 Excerpta Medica Amsterdam 1974
29 Pullman T N Lavender A R & Aho I Metabolism 16 358 1967
30 Quabbe H J Ramek W & Luyckx A S J Clin Endocrinol Metab 44 383 1977
31 Recant L Perrino P V Bhathena S J Danforth D N Jr & Lavine R L Diabetologia 12 319 1976
32 von Schenck H Clin Chim Acta 80 455 1977
33 — Acta Endocrinol (Kbh) (Suppl) 219 69 (Abstract) 1978
34 von Schenck H Larsson I & Thorell J I Clin Chim Acta 69 225 1976
35 Serrato M & Earle D P Proc Soc Exp Biol Med 102 701 1959
36 Soler N G Oates G D & Malins J M Proc R Soc Med 69 429 1976
37 Tiengo A Fedele D Marchiori E Nosadini R & Muggeo M Diabetes 25 408 1976
38 Trout D L Estes E H Jr & Friedberg S J J Lipid Res 199 1960
39 Unger R H & Raskin P Endocrinology vol II (ed V H T James) p 588 Excerpta Medica Amsterdam and Oxford 1975
40 Unger R D Parker M L & Daughaday W H J Clin Invest 41 25 1962
41 Valverde I Lemon H & Unger R H Diabetologia 11 381 (Abstract) 1975
42 Weir G C Horton E S Aoki T Slovik D Jaspan J & Rubenstein A H J Clin Invest 59 325 1977

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Recessive X-Linked Hyperuricemia with Gout and Renal Damage, Normal Activity of Hypoxanthine Phosphoribosyltransferase and Resistance to Azaguanine

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ABSTRACT A family is reported where four males have developed hyperuricemia, renal damage and, except for the youngest person affected gout at an early age The disease appears to be inherited as an X linked recessive metabolic error Clinically the patients have developed classical tophaceous gout before the age of 25 and have suffered repeated attacks of renal colic Renal tubular damage with decreased ability to concentrate and acidify urine was seen in a family member of only 16 years of age Progressive renal failure seems to develop slowly None in the family has shown neurologic symptoms, and two of the four affected men are apparently of at least average intelligence, two slightly below average One female carrier has repeatedly passed uric acid stones Studies of the red blood cell lysate have shown a normal activity of enzyme hypoxanthine phosphoribosyltransferase and an increased level of adenine phosphoribosyltransferase Skin fibroblasts from affected family members grew normally in the presence of 8-azaguanine Administration of azathioprine to the patients did not decrease their serum uric acid levels This is the first family described with this type of disorder of the purine metabolism

Key words hyperuricemia hypoxanthine phosphoribosyltransferase azaguanine resistant recessive X linked

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In 1967 Seegmiller et al (8) discovered that the almost complete lack of activity of the enzyme *hypoxanthine phosphoribosyltransferase* (HGPRT ase) (EC 2 4 2 8) previously though to be a dispensable salvage enzyme was the basis for the extreme hyperuricemia in the severe neurologic disease described by Lesch and Nyhan (6) The hyperuricemia of the patients suffering from this disease is resistant to azathioprine treatment probably as a consequence of their lack of HGPRT ase activity as this enzyme is considered necessary for the formation of the biologically active ribonucleotide of azathioprine

This report describes a family with apparently X linked recessive hyperuricemia gout before the age of 25 years and renal damage The fact that the affected members of the family did not respond to azathioprine treatment made us investigate the activity and some kinetic properties of HGPRT ase This is the first report of this type of metabolic disturbance occurring as an inherited disease

METHODS

Serum uric acid concentration was measured using a uricase method (7) Kidney concentrating capacity was measured as maximal urinary osmolality after the injection of pitressin tannate in oil (12) The renal acidification capacity was measured as the lowest pH attained and the ammonia excretion ability after oral ammonium chloride loading (13) Glomerular filtration rate was measured with the inulin clearance technique (9) or using single injection methods with ^{51}Cr EDTA (4) or inulin (1)

The urinary excretion of hypoxanthine and xanthine was determined according to the method described by Sorensen (10) The activity of HGPRT ase in erythrocytes and fibroblasts was determined according to the method described by Harris and Cook (5) Adenine phosphoribosyltransferase (APRT ase) (EC 2 4 2 7) was assayed using a similar method When the activity in fibroblasts was assayed thymidine triphosphate was added to the reaction mixture in order to minimize the effect of 5 nucleotidase

Resistance to 8-azaguanine was tested by growing fibroblasts from the patients and normal persons for six days in a medium containing 8-azaguanine The concentrations used are given in Fig 2 The cells prior to and after the incubation period were counted and the result expressed as the percentage of surviving cells

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Abbreviations HGPRT-ase=hypoxanthine phosphoribosyltransferase APRT ase=adenine phosphoribosyl transferase

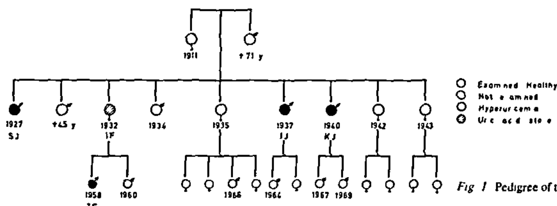


Fig 1 Pedigree of the family

CASE REPORTS

The medical histories of the three brothers, cases 1, 2 and 3, are very similar and given in detail only for patient 1. The pedigree is presented in Fig. 1.

Case 1 (S. J.)

A 51 year-old truck driver, married, with an unremarkable clinical history except for gout and nephropathy. His growth and early development were normal. Mentally he appears to be normal although somewhat withdrawn and of an intelligence probably slightly below average. No tendency for self mutilation has been observed. At the age of 25 years acute pain, tenderness and swelling developed in the left heel. During the next five years he suffered from frequent attacks of pain and swelling in several joints, particularly the ankles and the metacarpophalangeal joints. At the age of 28 years he was diagnosed as suffering from gout. Treatment with probenecid was started and resulted in a lower attack rate. Eight years later hematuria was noticed for the first time. In 1966, at the age of 38, he was investigated for the first time at Sahlgren's Hospital.

At the clinical examination several tophi were found at both ears and on the finger joints, the largest being plum sized. BP was 180/110 mmHg. The physical examination was otherwise unremarkable. Serum creatinine was 150 μmol/l, serum uric acid 624–786 μmol/l.

Microscopy of the urinary sediment was repeatedly normal. A small quantity of protein, less than 1 g/l, was generally found in the urine. I.v. urography showed both kidneys to be small, about 11 cm long, with blunting of the calyces. The picture was similar to that seen in papillary necrosis. Urine culture was sterile. Inulin clearance was 52 ml/min and PAH clearance 361 ml/min, giving a filtration fraction of 0.16. The investigations of the kidney function are summarized in Tables I and II.

The patient was treated with allopurinol 500 mg daily. His serum uric acid decreased to 234 μmol/l and remained at a low level at subsequent controls.

His renal function then slowly deteriorated and in March 1973 the inulin clearance was 34 ml/min/1.73 m². The serum uric acid was frequently elevated. It was found that the patient had not taken his allopurinol regularly. The inadequate treatment may have contributed to the deterioration of renal function.

From 1972 until Aug. 1974 the patient was treated with a very high dose of allopurinol, 900 mg/day, in spite of his renal insufficiency. This medication did not produce any side-effects, the bone marrow smear and routine hematologic values being unremarkable. Serum uric acid

Table I. *Laboratory investigations*

Case no	Serum uric acid highest (μmol/l)	Urinary uric acid (μmol/24 h)	Hypoxanthine xanthine ratio urine	HGPRTase in RBC lysate*	APRTase in RBC lysate*
1	1 080	4.8		60.4	43.7
2	888	5.9		66.3	44.3
3	870	5.9	2:1	69.0	43.9
4	780	3.6	2:1	67.2	46.7
Reference value	150–450		1:10	50.9–70.6	18.6–23.3

* Expressed as nmol product/mg protein/60 min
Fibroblasts were azaguanine resistant in all cases

Table II *Urinary excretion of titrable acid and ammonia during acid load*

Case no	Inulin clearance (ml/min/1 73 m²)	Max conc (mOsm/ kg water)	Lowest pH at test	Titrable acid (nmol/min)	Ammonia (μmol/min)
1	51	462	5 5	20	35
2	58	530	5 5	20	25
3	58	629	5 5	25	29
4	88	627	5 7	26	32
Reference value	124±24	>800	4 6–5 5	24–51	33–75

has remained below 120 μmol/l and the patient is in good health The allopurinol dosage has been decreased to 300 mg/day The renal function is stable no attacks of gout have occurred since 1972 and the tophi have completely disappeared

In 1973 the allopurinol therapy was interrupted for ten days and azathioprine 200 mg/day was given During this period the serum uric acid concentration increased rapidly from 528 to 1 080 μmol/l The urinary excretion of uric acid did not decrease during azathioprine treatment The results of the investigations of purine metabolism are shown in Table I

Case 2 (I J)

The second brother now 41 years old is apparently of average intelligence and has had no history of psychiatric or social difficulties From the age of 14 to 18 years he was repeatedly hospitalized because of hematuria frequently with sharp pain From the age of 21 to 27 years he suffered from attacks of acute gout particularly as podagra in spite of treatment with sodium bicarbonate and probenecid Allopurinol treatment was initiated in 1965 and the patient has been free from gout since Azathioprine 150 mg daily was given instead of allopurinol for 14 days During this time the serum uric acid values increased from 240 to 684 μmol/l I v urographies in 1964 and 1972 showed kidneys of normal size but with blunting of the calyces Relevant observations at physical examination and laboratory investigation are summarized in Tables I and II

Case 3 (K J)

A 38 year old factory worker who in 1973 was pensioned primarily because of psychiatric problems He suffers periodically from anxiety and has received out patient psychiatric care for several years He seems to be of dull normal intelligence and has never shown any remarkably aggressive behaviour or evidence of self mutilation A full scale IQ test has given a value of 100 while a more verbal test gave an IQ of 90 From the age of 16 years he has experienced attacks of gout and has been hospitalized repeatedly because of renal colic with hematuria. Unfortunately no kidney stone has ever been analyzed Because of the psychiatric problems the patient has not quite regularly taken the allopurinol he has been prescribed since 1965 and has suffered from gout attacks occasionally even in recent years I v urographies in 1965 and 1972 showed kidneys of normal size but with evidence of papillary necrosis When azathioprine 200 mg/day was substituted for allopurinol during an eight-day trial period serum uric acid increased from 480 to 840 μmol/l

Case 4 (T F)

This 16 year-old boy was admitted to our hospital in June 1974 as participant in the family study His medical and developmental history were unremarkable except for a hospitalization for infectious hepatitis at the age of 7 The boy has never shown evidence of mental or social abnormality and is clearly of at least average intelligence

Serum uric acid measured for the first time at the age of 15 because of his family history was 594 μmol/l and two months later 780 μmol/l The investigation gave evidence of a slightly decreased glomerular filtration rate with an inulin clearance of 88 ml/min/1 73 m² BSA a urinary concentration defect and a decreased ability to excrete ammonia during an acid load I v pyelography was normal His serum uric acid level normalized on allopurinol treatment 300 mg daily He has never suffered from an attack of gout

Case 5 (I F) obligatory carrier

This 47 year-old woman was first examined at this hospital at the age of 33 and was found to have a serum uric acid value of 252 μmol/l From the age of 26 to 38 she suffered from about ten attacks of renal colic One stone was analyzed and it contained only uric acid Serum uric acid measured twice during the last years showed values of 348 and 366 μmol/l She has never suffered from gout and appears to be of at least average intelligence

The younger brother of case 4 has also been investigated His serum uric acid level is normal and the activities of HGPRT ase and APRT ase in red blood cell lysates prepared from his blood are normal As shown in Fig 1 four other family members—the mother of the affected three brothers one asymptomatic brother one asymptomatic sister and her son—have also been examined and found to be free from the disease The investigations revealed a negative history of gout and renal colic normal levels of serum uric acid and normal activities of HGPRT ase and APRT ase

The mother of the hyperuricemic brothers has a good knowledge of the family—to some extent more than two generations back She is not aware of any case of gout or mental deficiency in the family except her three gouty sons described above

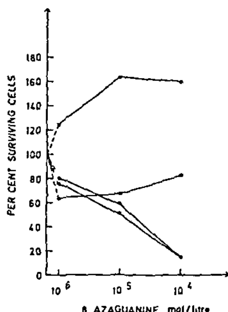


Fig 2 Survival of fibroblasts grown in medium containing 8-azaguanine O—O = Controls ●—● = patients

RESULTS

The ability to excrete an acid urine after ammonium chloride loading was studied in all afflicted males The results are given in Table II together with the glomerular filtration rate and the maximal urinary concentration after injection of pitressin tannate in oil

A decreased concentration ability is evident out of proportion to the reduction of glomerular filtration rate The ability to produce a maximally acid urine after a load of ammonium chloride is decreased in all patients Even at the reduced level of glomerular filtration in these patients the ability to obtain a low pH is decreased compared to e g patients with non-obstructive pyelonephritis The ability to excrete ammonia is reduced in approximate proportion to the decreased ability to acidify the urine if compared to the nomogram of Wrong and Davies (13)

The uric acid clearance is normally dependent upon the glomerular filtration rate the diuresis and the serum uric acid level making an interpretation of the values given in Table II difficult It is clear however that the hyperuricemia in our patients is not caused by a decreased ability of the kidney to clear uric acid from the blood

The activities of HGPRT ase in erythrocytes and cultured fibroblasts from the patients were all within the reference range of controls analyzed at the same time (Table I) On the other hand the activities of APRT ase were about twice as high in erythrocytes from the patients than from controls (Table I) Case 5—the mother of case 4 and an obligatory carrier—had also a raised APRT ase activity

Enzyme prepared from the patients erythrocytes had the same apparent K_m for hypoxanthine adenine and phosphoribosyl pyrophosphate as enzyme prepared from erythrocytes of the controls Preliminary studies using 8 azaguanine as substrate have not revealed any difference in affinity between enzymes prepared from patients and controls

The patients excreted increased amounts of hypoxanthine the hypoxanthine xanthine ratio being about 2 1 whereas controls excreted considerably more xanthine than hypoxanthine

Fibroblasts from the patients grown in medium containing 8 azaguanine showed no growth inhibition at drug concentrations which caused fibroblasts from controls to show a marked inhibition (Fig 2)

DISCUSSION

The clinical manifestations in the affected three brothers of this family are quite uniform Severe tophaceous gout with the first attacks occurring before the age of 25 repeated attacks of renal colic presumably because of the passage of uric acid stones and some degree of renal insufficiency Two brothers are possibly slightly below average in intelligence No signs of neurologic disease and no clearly pathological personality traits have been observed in any member of the family In all subjects investigation has shown pronounced hyperuricemia defects in the ability of the kidneys to concentrate and acidify urine and absence of effect of azathioprine on the level of serum uric acid Allopurinol has been uniformly effective in lowering the uric acid level and has also reduced or abolished the gout attacks

It is apparent from the family tree that the most likely mode of inheritance of the disease in this family is as an X linked recessive trait This concept is supported by the finding of a raised level of APRT ase in the erythrocyte lysates from the presumed female carrier Also in the Lesch Nyhan syndrome heterozygous carriers show this enzyme

abnormality She also has given evidence of the disease by the passage of uric acid stones a highly unusual history in a female The history of case 1 (S J) who received late and insufficient treatment probably resulting in renal insufficiency shows that all affected males in the family should be treated as early as possible Case 4 (T F) showed evidence of renal tubular dysfunction and slightly decreased glomerular filtration already at the age of 16 years The question arises whether this reflects damage from hyperuricemia or an associated renal disorder *Investigation of still younger affected members of* the family could provide an answer to this question but no other family member is presumed to be affected with the disease provided it is inherited as an X linked recessive disorder The kidney function was decreased in all four individuals studied measured both as glomerular filtration rate and through tests of tubular function As is evident from Table II the ability to attain a maximally low pH during *an acid load is marginally decreased and the maxi*mal excretion of ammonia is low In this respect the patients are similar to other patients with renal tubular damage in chronic pyelonephritis and uric acid nephrophathy

The nature of the biochemical defect remains to be clarified It seems probable that these patients have a mutant form of HGPRT ase Benke et al (2) have reported that addition of non toxic amounts of magnesium to fibroblast cell cultures made fibroblasts from Lesch Nyhan strains sensitive to 8 azaguanine One of our patients (T F) was tested in this respect but showed no increased sensitivity to 8 azaguanine on the addition of magnesium to the cell culture medium One possibility that has not been investigated is that the enzyme HGPRT ase is not fully active in vivo in spite of its normal activity in vitro If this is the case all the biochemical abnormalities observed in our patients can be explained but the cause of the resistance to 8 azaguanine of the fibroblasts in tissue culture is not clear

Biochemically our patients are similar to patient C M reported by Sorensen and Benke (11) and Benke and Herrick (3) That boy also showed a normal HGPRT ase activity and an increased APRT ase activity in his red blood cell lysates and resistance to azaguanine of fibroblasts in culture Their patient seems to have been more severely afflicted with a serum uric acid level of 1980 μmol/l while the highest value in any of our patients is 1090 μmol/l Patient C M also showed mild neurological symptoms and at one instance self mutilating behaviour which has never occurred in the patients of the family described by us

REFERENCES

1 Alestig K Hood B & Vikgren P Inulintest Lakartidningen 63 1554 1966
2 Benke P J Herbert A & Herrick N In vitro *effects of magnesium ions on mutant cells from pa*tients with the Lesch Nyhan syndrome N Engl J Med 289 446 1973
3 Benke P J & Herrick N Azaguanine resistance as a manifestation of a new form of metabolic over production of uric acid Am J Med 52 547 1972
4 Brøchner Mortensen J A simple method for the determination of glomerular filtration rate Scand J Clin Lab Invest 30 271 1972
5 Harris H & Cook P R The synthesis of an enzyme determined by an erythrocyte nucleus in a hybrid cell *J Cell Sci 5 121 1969*
6 Lesch M & Nyhan W L A familial disorder of uric acid metabolism and central nervous system function Am J Med 37 561 1964
7 Pretorius E & Poulsen H Enzymatic determination of uric acid with detailed directions Scand J Clin Lab Invest 5 273 1953
8 Seegmiller J E Rosenbloom F M & Kelley W N Enzyme defect associated with a sex linked human neurological disorder and excessive purine synthesis Science 155 1682 1967
9 Smith H S The kidney Structure and function in health and disease Oxford University Press New York 1951
10 Sorensen L Mechanism of excessive purine biosynthesis in hypoxanthineguanine phosphoribosyl transferase deficiency J Clin Invest 49 968 1970
11 Sorensen L B & Benke P J Biochemical evidence for a distinct type of primary gout Nature (Lond) 213 1122 1967
12 de Wardener H E Vasopressine tannate in oil and the urine concentration test Lancet 1 1037 1956
13 Wrong O & Davies E F The excretion of acid in renal disease Q J Med 28 259 1959

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Renal Transplantation in Amyloidosis

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ABSTRACT During a three year period renal transplantation was performed in 12 patients with amyloidosis This disease was primary (or the cause unknown) in two cases and secondary in ten In the latter cases the primary disease was rheumatoid arthritis in six ankylosing spondylitis in one, osteomyelitis in two and tuberculosis in one Five of the 12 patients were alive one year after transplantation Two years after transplantation four out of seven were alive Graft survival was the same At the end of the three year period five patients were alive In two of these cases renal biopsy showed amyloid deposits in the transplant two and three years, respectively, after the transplantation

Key words amyloidosis renal transplantation

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Previously it was considered more or less a condition for renal transplantation that the recipient suffered from a primary renal disease while patients with renal failure secondary e g to diabetes amyloidosis or SLE were not considered for this kind of treatment During the last few years a change has occurred on this point The altered attitude to the selection of candidates for renal transplantation was reflected in the 1972 report of the European Dialysis and Transplant Association (4) and the trend has persisted

This paper reports the results of renal transplantation in patients with amyloidosis

PATIENTS AND METHODS

The series consists of 12 patients with amyloidosis transplanted during the period Sept. 5 1973–Aug 10 1976 The time of observation is at least one year The total number of patient months is 181 During the same period a total of 340 necrokidney transplantations were performed (5 131 patient months) The proportion of patients with amyloidosis is 3 5%

Eight of the recipients were on haemodialysis treatment before the transplantation Other details relating to the series appear in Table I

Only necrokidneys were used The immunosuppression therapy consisted of methylprednisolone in decreasing doses so that the maintenance dose about half a year after transplantation was 8–16 mg every second day Azathioprine was in general given in a dose of 150 mg daily depending on the leukocyte and thrombocyte values and the liver function

Amyloidosis was diagnosed by renal and/or rectal biopsy in 10 cases in 3 of these also by fine needle aspiration biopsy of the spleen (16) Biopsy was performed on average one year after the appearance of the first clinical signs of amyloidosis (proteinuria with or without nephrotic syndrome elevated serum creatinine) In two patients who died one and two months after the transplantation the diagnosis was established at autopsy The specimen was fixed in formalin or glutaraldehyde stained with alkaline Congo red and examined in polarized light for green birefringence

Fine needle biopsy of the transplant was performed on five surviving patients in order to check the possible presence of amyloid deposits by electron microscopy (17) Conventional percutaneous renal biopsy was performed in two cases on the suspicion of rejection nephritis or amyloidosis (proteinuria impaired renal function)

RESULTS

Patient survival and graft survival are shown in Table II Seven patients died one to four months after transplantation The 12 recipients developed a total of 14 different complications (infectious in 8 cardiovascular in one gastrointestinal in 4 and psychiatric in one) or 0 08 complications per patient month The causes of death and the distribution of amyloidosis in different organs and intrarenally appear in Table III At autopsy amyloid was not found in the transplant in any case

The general condition of four of the five survivors was good and their renal function was normal or almost normal In one of them a leg amputation had to be performed owing to gangrene One showed marked proteinuria and impairment of the general condition her renal function was markedly reduced and haemodialysis treatment proved necessary about two years after the transplant it

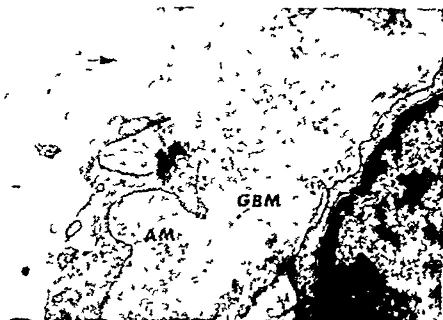


Fig 1 Electron micrograph of kidney aspiration biopsy showing abundant glomerular amyloid fibrils located mainly subepithelially (AM) and extending into the urinary space (arrow) GBM = glomerular basement membrane × 14 235

Membranous glomerulonephritis in the transplant was demonstrated by percutaneous renal biopsy while staining for amyloid was negative Three survivors did not excrete protein whereas a positive finding was made in one besides the above mentioned patient In the case in question nephrotic syndrome developed three years after a successful transplantation and amyloid was demonstrated in the transplant by both fine needle aspiration biopsy and percutaneous renal biopsy

CASE REPORT

A previously healthy 37 year-old woman developed nephrotic syndrome in the autumn of 19?? She was treated with corticosteroids but this treatment proved ineffective In Jan 1973 renal needle biopsy showed glomerular amyloidosis on Congo red staining but biopsies of the liver and the rectum were negative Extensive examinations failed to reveal any underlying chronic disease Hence the amyloidosis was considered "primary"

Her renal function deteriorated and in March 1974 when serum creatinine was 955 µmol/l (normal range 60–110) a necrokidney of A match was transplanted Immediate diuresis ensued and serum creatinine fell to 165 µmol/l in six days An episode of mild rejection responded to high doses of methylprednisolone and serum creatinine was normal three weeks after the transplantation Total serum protein reached normal values in July 1974 but some degree of proteinuria persisted until Nov 1974 after which it disappeared.

Table I *Clinical data on the patients*

	No of pats	Years Mean	Years Range
Males	8		
Females	4		
Histocompatibility			
A-matches	2		
B-matches	2		
C matches	4		
D-matches	4		
Diagnosis			
Secondary amyloidosis	10		
Principal underlying disease			
Rheumatoid arthritis	6		
Ankylosing spondylitis	1		
Osteomyelitis	2		
Tuberculosis	1		
Primary (or unknown) amyloidosis	2		
Alive (follow-up 12–42 mo)	5		
Dead (1–4 mo after transplantation)	7		
On haemodialysis before transplantation (0 5–13 mo)	8		
Age at transplantation		48 6	31–64
Age at first signs of renal amyloidosis		44 3	28–63
Duration from onset of primary disorder to first signs of renal amyloidosis	10	13 4	1 ?7

Table II *Patient survival and graft survival after renal transplantation*

	3 mo	6 mo	1 y	2 y	3 y	4 y
Patient survival	7/12	5/12	5/12	4/7	2/2	1/1
Graft survival	6/12	5/12	5/12	4/7	2/2	1/1

The patient did well and had normal renal function tests and urinary findings through 1975 and 1976 In Dec 1976 slight proteinuria was observed In April 1977 she developed moderate lower limb oedema and proteinuria 12 g/day and a slight rise in serum creatinine was noted At this stage fine needle aspiration biopsy of the transplant revealed abundant amyloid deposits in the glomeruli (Fig 1) During the summer of 1977 the nephrotic syndrome was aggravated and serum creatinine reached 300 μmol/l in August A conventional needle biopsy of the transplant showed Congo red positive amyloid deposits in the mesangia of seven out of ten glomeruli while the remaining three were completely destroyed All vessels had thickened walls infiltrated by amyloid

In one more case electron microscopic investigation of a specimen of the transplant obtained by aspiration biopsy revealed amyloidosis

DISCUSSION

In occasional cases regression of amyloidosis has been observed in patients with the secondary form of the disease (12 14 18 19 20) A prerequisite for this outcome is that the primary disorder (tuberculosis osteomyelitis sepsis etc) is cured The results of treatment of amyloidosis with melphalan (8) have not been convincing while somewhat more favourable results have been reported with heparin (13) and dimethyl sulfoxide (15)

In general the disease is not regressive and therapy has little or no effect Active treatment of uraemia must therefore be resorted to in the terminal stage The long term results of chronic dialysis treatment of terminal uraemia due to amyloidosis have been discouraging (7) According to the European report the two-year survival on hospital dialysis is 23% poorer in amyloidosis patients than in the total material (all renal patients) (5)

The first renal transplantations in patients with amyloidosis were performed about ten years ago (1 3) Since then the number has increased (7 9) The results have in general been poorer than for patients with other primary renal disease though more favourable than the results of dialysis treatment of renal amyloidosis

In our 12 patients the results with regard to patient and graft survival are in accordance with those reported in more extensive amyloidosis series (9) The seven deaths occurred relatively early after transplantation Most complications were infectious a finding that disagrees slightly with our total necrokidney transplantation series in which infectious and cardiovascular complications were equally common (11) Similar figures for the complications and causes of death were reported in a total series of patients with various renal diagnoses published by other authors (2)

At autopsy of the seven patients who died

Table III *Causes of death and postmortal findings in seven renal transplant recipients with amyloidosis*

	No of pats
Cause of death	
Sepsis	4
Gastrointestinal bleeding	1
Cardiac infarction	1[a]
Pneumonia	*1*
Distribution of amyloid in various organs	
Kidney	7/7
Spleen	7/7
Liver	7/7
Rectum	4/4
Heart	6/7
Thyroid	5/6
Pancreas	4/5
Lung	2/7
Transplant	0/7
Site of amyloid deposits in the kidneys	
Glomeruli	7/7
Arterioli	7/7
Tubuli	5/7
Interstitial tissue	5/7

Two with functioning graft [a] With functi

amyloid deposits were not found in the transplant This may however be due to the fact that all these deaths occurred shortly after transplantation On the other hand the distribution of amyloid in different organs was in agreement with previous findings (10 12)

In two of five cases followed up from one to four years amyloid was demonstrated in the transplant two and three years respectively after a successful renal transplantation This seems to indicate that recurrence of the disorder in the transplant in amyloidosis patients is relatively common after renal transplantation Similar indubitable amyloid recurrences have already been described in some cases (6 7 9) which is remarkable if one considers the relatively small number of transplantations hitherto performed in amyloidosis

In spite of the fact that amyloidosis is a systemic disease sometimes associated with severe cardiovascular and infectious complications and despite the relatively high incidence of recurrence in the transplant after some years renal transplantation is today considered the best therapeutic measure that can be offered an amyloidosis patient with terminal uraemia

REFERENCES

1 Belzer F Ashby B Gulyassy P & Powell M Successful seventeen hour preservation and transplantation of human-cadaver kidney N Engl J Med 278 608 1968

2 Brynger H Bitter Suermann H Gabel H Ahlmén J Blohmé I Gustafsson Å & Gelin L E Complications after renal transplantation Scand J Urol Nephrol (Suppl) 38 113 1977

3 Cohen A Briccetti A Harrington J & Mannick J Renal transplantation in two cases of amyloidosis Lancet 2 513 1971

4 Gurland H Brunner F v Dehn H Harlen H Parsons F & Scharer K Combined report on regular dialysis and transplantation in Europe III 1972 Proc Eur Dial Transplant Assoc 10 XVII 1973

5 Gurland H Brunner F Chantler C Jacobs C Scharer K Selwood N Spies G & Wing A Combined report on regular dialysis and transplantation in Europe VI 1975 Proc Eur Dial Transplant Assoc 13 3 1976

6 Jones M Adams J & Passer J Amyloidosis in a renal allograft in familial mediterranean fever Ann Intern Med 87 580 1977

7 Jones N Renal amyloidosis pathogenesis and therapy Clin Nephrol 6 459 1976

8 Jones N Hilton P Tighe J & Hobbs J Treatment of primary renal amyloidosis with melphalan Lancet 2 616 1972

9 Kennedy C & Castro J Transplantation for renal amyloidosis Transplantation 24 382 1977

10 Kuhlback B Renal biopsy in the diagnosis of amyloidosis In Amyloidosis (ed O Wegelius & A Pasternack) p 387 Academic Press London 1976

11 Kuhlback B Wallenius M & Kock B Complications after renal transplantation In preparation

12 Kuhlback B & Wegelius O Secondary amyloidosis A study of clinical and pathological findings Acta Med Scand 180 737 1966

13 Lindqvist B & Isaksson B Heparin treatment in renal amyloidosis Abstr XI Congress of the European Dialysis and Transplant Association 13 1974

14 Lowenstein J & Gallo G Remission of the nephrotic syndrome in renal amyloidosis N Engl J Med 282 128 1970

15 Osserman E Isobe T & Farangi M Effect of dimethyl sulfoxide (DMSO) in the treatment of amyloidosis In Amyloidosis (ed O Wegelius & A Pasternack) p 553 Academic Press London 19 6

16 Pasternack A Fine needle aspiration biopsy of spleen in diagnosis of generalized amyloidosis Br Med J 2 20 1974

17 Pasternack A Helin H Törnroth T Rantala J Vaisanen J & Rahka R Aspiration biopsy of the kidney with a new fine needle A way to obtain glomeruli for morphological study Clin Nephrol 10 79 1978

18 Reimann H Recovery from amyloidosis JAMA 104 1070 1935

19 Triger D & Joekes A Renal amyloidosis—a fourteen year follow up Q J Med 42 15 1973

20 Waldenström H On the formation and disappearance of amyloid in man Acta Chir Scand 63 479 1928

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Paraplegia in Myelomatosis—A Study of 20 Cases

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ABSTRACT A retrospective study of 20 cases of multiple myelomatosis and paraparesis treated during 1966–77 is presented All patients but one had been submitted to decompressive laminectomy A close correlation between duration of paraparesis before operation and prognosis was found eight patients with a duration of paraparesis of several days remaining paraplegic No particular paraparesis-prone patient group with respect to duration of myelomatous disease Ig class or various laboratory findings could be identified In several instances radionuclide scan was effective in localizing vertebral lesion In most patients radicular type back pain signalled vertebral damage and subsequent paraparesis In many cases long survival after operation and excellent to good functional results made surgery a worthwhile procedure The importance of postoperative radiotherapy and adequate chemotherapy is stressed

Key words myelomatosis paraplegia neurosurgery

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Multiple myelomatosis usually carries a poor prognosis though modern treatment with cytostatics such as alkeran alone or in combination with corticoids (2 8 15) has improved the prognosis considerably A prolonged survival on cytostatic therapy could be associated with an increase in complications but the incidence of paraparesis does not seem to have changed with the introduction of modern chemotherapy—approximately 10% of patients with multiple myelomatosis are reported to show paraparesis (4 16)

During the past 11 years we have treated 20 patients with multiple myeloma and paraparesis at the Departments of Medicine and of Neurosurgery University Hospital Linkoping Sweden We were impressed by the fact that the diagnosis was often made too late in these patients to enable successful neurosurgical treatment of the paraplegic state We therefore studied these 20 patients retrospectively to see whether we could identify a particular risk group among multiple myelomatosis patients prone to develop paraparesis The variables that we felt could be of interest were serum or plasma levels of immunoglobulins calcium albumin creatinine urea and Hb and urinary light chain excretion (Bence Jones protein) This investigation seemed the more useful since nothing similar had been done previously In addition to efforts to identify a paraparesis risk group in multiple myelomatosis we also hoped to be able to define symptoms signalling imminent paraparesis by analysing symptoms and signs that occurred in our patients

It is extremely important that myelomatosis patients with paraparesis receive neurosurgical treatment in time if a good functional result is to be achieved (4 17) if complete paralysis of paraplegia is allowed to persist for more than approximately 24 hours it will generally be irreversible

PATIENTS

Twenty patients with myelomatosis and paraparesis were studied 14 men and 6 women At the time paraparesis developed the age for men was 43–75 years (mean 63) and for women 47–71 years (mean 62)

The study is retrospective and includes all myelomatosis patients referred to our Neurosurgical Unit during the past 11 years Most patients were treated by decompressive laminectomy

Myelomatosis was defined as follows 1) M-component in plasma and/or urine 2) Increased number of bone marrow plasma cells with considerable atypia 3) Lytic bone lesions on X ray and/or positive radioisotope skeletal scan 4) Histological confirmation of myelomatous condition in operation specimen

One patient fulfilling the above criteria was given the diagnosis of light chain disease (19) Another was classified as having non secretory myelomatosis (18) since no M-component was present in plasma or urine

Six of the 20 patients with myelomatous disease developed paraparesis as the first symptom The total observation time varied from 6 months to 12 years 3 years

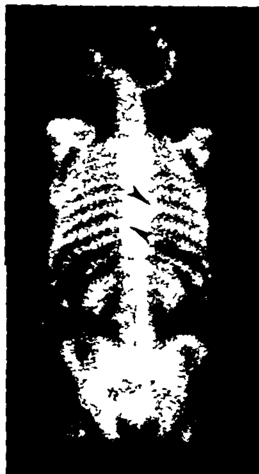


Fig 1 Skeletal scintigraphy using ^{99m}technetium diphosphonate Increased uptake of the radionuclide is seen in two vertebral bodies (*arrows*) several ribs one shoulder denoting an area of accelerated skeletal

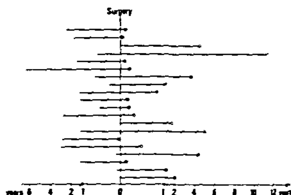


Fig 2 Total duration of illness (mean 38 months range 9–143) and postoperative survival (mean 22 months range 0–136) ● = Dead O = alive at the time of the study

METHODS

Agarose gel electrophoresis of plasma and urine concentrates was done as described by Laurell (10)

Immunoglobulins were determined by the nephelometric technique of Lizana and Hellsing (13) or by the Mancini technique (14) The total immunoglobulin of each class was measured both monoclonal and polyclonal the M-component not being measured separately Reference quantities in normal individuals for individual immunoglobulins were IgG 7 0–15 0 g/l IgA 1 2–3 0 g/l IgM 0.5–1.8 g/l)

Detection of Bence Jones proteinuria

Freshly voided urine was concentrated with the aid of Minicon® B 15 cells (Instrument AB Lambda Stockholm Sweden) (11) The degree of concentration varied with the urinary protein concentration samples with low protein content being more concentrated than samples with high protein content Total protein and albumin concentrations in untreated urine specimens were determined by the biuret (6) and nephelometric (13) methods respectively The urine concentrate was then examined by agarose gel electrophoresis In most instances only two or three protein fractions could be seen namely albumin and a monoclonal protein (band) in the gammaglobulin region. *Given the total protein and albumin concentrations of the* urine sample a rough quantitative estimate of urinary immunoglobulins can be obtained

Immunoelectrophoresis was used to classify M-components in both plasma (serum) and urine concentrates with respective immunoglobulin (heavy chain) class and light chain group

Bone marrow specimens were obtained from the iliac crest or sternum using conventional technique Smears and sections were studied after staining with haematoxylin-eosin or Giemsa and the morphological pattern was defined Plasma cell abundance and degree of atypia were noted in particular

X ray studies included skeletal survey with particular attention to the vertebral column Tomography was often used to obtain detailed information on a particular vertebra In 12 cases contrast myelography (metrizamide (14) since 1975) was done preoperatively to obtain details of the localization and extent of the lesion

Radionuclide skeletal scanning was used in five instances I v injection of 15 mCi ^{99m}technetium-diphosphonate (9) was followed after 4 hours by scanning of the entire skeleton with a gamma-camera (Maxi-camera, General Electric Chicago) Increased uptake of the radionuclide is easily detected and indicates increased skeletal metabolism in the region concerned Fig 1 shows a skeletal scintigram

RESULTS

In 6 of 20 patients with myelomatosis complicated by paraparesis paraparesis was the first symptom to appear In the others the initial symptoms varied and included lassitude pain in arm and shoulders and pathological fractures (in 3 cases) In 9 patients

Table 1 *Correlation between prognosis and preoperative duration of paraparesis*

Prognosis	Preoperative duration of symptoms		
	<24 h	1–4 d	>4 d
Persisting complete paraplegia	0	4	4
Partial disappearance of paraplegia	2	3	1
Complete disappearance of paraplegia	6	0	0

One patient died prior to surgery

the paraparesis was preceded by pain in the thoracic or lumbar spine The interval from the first clinical diagnosis of myeloma to the development of paraparesis was 0–75 months (mean 19) (Fig 2)

Careful scrutiny shows that before the development of paraparesis 14 of 20 patients (70%) had back pain in one form or another Characteristic radicular pain that is burning pain on one or both sides in the segment corresponding to the vertebral lesion was present in 13 of them one patient complained of uncharacteristic pain in the thoracic spine radiating to the shoulder In six patients the paraparesis developed without previous pain but was accompanied by decrease in sensory function as shown by neurological examination In 11 patients the sensory or motor (1 case) symptoms persisted for more than one month before the diagnosis was established and surgery done

There was a close correlation between duration of paraparesis before operation and prognosis of 8 patients in whom the paraparesis was present for less than 24 hours it disappeared completely in 6 and partly in 2 of 8 patients with paraparesis for 1–4 days (4 cases) or more than 4 days (4 cases) it persisted after operation (Table 1)

All vertebral lesions causing paraplegia were located to the thoracic spine except for one in the lumbar spine Fig 3 shows the uniform spread of lesions within the thoracic spine

No consistent change in ESR or Hb concentration could be found many patients showed moderate to severe anaemia indicating advanced myelomatous disease—in others the Hb concentration was normal

Sixteen patients showed an IgG M component—two had IgA myeloma one was classified as having light chain disease (19) and one as having non secretory myeloma (18) In the latter patient immunofluorescent studies of the bone marrow (performed by T Skogh Department of Medical Microbiology) revealed kappa chains within numerous plasma cells Individual M components and immunoglobulin quantitation are classified in Fig 4

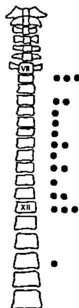


Fig 3 Distribution of lesions within the spinal column Each dot indicates the vertebral lesion in a single patient

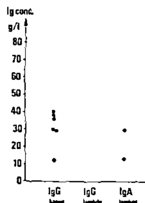


Fig 4 Concentration of plasma IgG and IgA in individual patients (●) patients with IgG M-components bearing kappa or lambda light chains Diagonal markings indicate normal ranges

Non involved polyclonal ("background) immuno globulins were depressed in most instances

Renal function measured by serum creatinine and urea serum calcium concentrations and alkaline phosphatase were determined but did not seem to deviate from normal more often than in myeloma patients without paraparesis

The occurrence of Bence Jones protein was recorded by a semiquantitative technique described previously (12) Ten patients showed concentrations of less than 50 mg/l and 8 of 100 mg/l–1 g/l In only two patients did the concentration exceed 1 g/l (1 5 and 9 g/l)

In 8 patients plain X ray studies of the spine including tomography were sufficient to confirm the localization of the lesion suspected after neurological examination and neurosurgical procedure was therefore undertaken without further X ray studies In 12 patients however positive contrast myelography was required to obtain definite information regarding the localization of the spinal cord compression It was found that myelography always confirmed the localization of spinal cord injury indicated by the plain X ray examination

Radionuclide skeletal scanning using ^{99m}Tc diphosphonate (9) was carried out in 5 patients in whom back pain was a prominent symptom In all of them increased uptake took place at the level at which compression causing medullary damage subly occurred X ray examination 2–6 weeks er the scanning disclosed no evidence of skeletal damage but on reexamination after the development of paraparesis the bone lesions were demonstrated Thus in these 5 patients radionuclide scanning seemed to be more effective than plain X ray for demonstrating early myelomatous engagement of vertebral bodies leading to paraparesis

Bone marrow studies were performed in all cases to confirm the diagnosis They showed increase in plasma cells many with malignant features such as chromophobe nucleoli multiple nuclei and so forth Surprisingly no tendency to sheet like growth of the plasma cells was observed

Treatment

Nineteen patients received neurosurgical treatment Decompressive laminectomy was done the tumour arising from the vertebral body and compressing the medulla being exposed and removed One patient was too poorly for operation In all cases histological examination showed poorly differentiated myeloma and incomplete removal

Eighteen of the 20 patients received postoperative irradiation to the operation area since surgery had not been radical to prevent recurrence of medullary compression and to reduce pain (5) One patient died postoperatively before irradiation was started and another before operation

In 14 patients the diagnosis of myelomatosis was made at some time before the paraparesis and in all of these cases cytostatic therapy was then given At least 8 patients received intermittent melphalan–corticoid treatment as advocated by Alexanian et al (2) and 3 others received continuous low-dose melphalan therapy (15) One patient was given intermittent cyclophosphamide therapy (3) Two received intermittent treatment with doxorubicin/C C N U (1) after relapsing on melphalan/corticoid therapy

DISCUSSION

This retrospective survey of 20 patients with myelomatosis and paraparesis indicates that radicular thoracic pain often precedes the motor or sensory symptoms of paraparesis by several months, but it was frequently not recognized as a warning sign Further slowly developing motor paralysis in the legs has often failed to lead to neurosurgical measures until too late commonly owing to ignorance of the risks involved We therefore want to emphasize the view put forward by Cohen and Rundles (4) and Snapper and Kahn (17) it is of extreme importance prognostic and otherwise in patients with myeloma localized to the vertebral column to note symptoms indicating nerve root compression (such as radicular pain) as they frequently are forerunners of paraparesis It is also of extreme importance to perform decompressive neurosurgery on the appropriate level of the spine as soon as possible after symptoms of paraparesis have occurred The data presented in Table I illustrate this point very well

In order to achieve efficient neurosurgical decompression it is essential to establish accurately the localization of the lesion In most instances this can be done from the history careful neurological examination and thorough X ray examination of the cervical and thoracic spine With good X ray technique it is usually possible to demonstrate even small destructions of the pedicles In one patient

conventional X ray failed to visualize the 7th cervical vertebra but tomography showed total compression We feel that myelography is indicated only when the neurological and X ray findings dis agree or when plain X ray fails to show the site of the lesion When myelography is required we prefer the positive contrast technique (7)

With the ^{99m}Tc-diphosphonate scan we were able to establish the presence of changes in the spine in 5 cases at sites where myelomatous collapse of ver tebrae later occurred causing paraparesis In such cases therefore radionuclide scan is apparently superior to conventional X ray for localization of early myelomatosis of the spine No conclusions can be drawn from this limited series however Numerous recent reports indicate that radionuclide scan using various isotopes may be less informative than conventional X ray in myeloma (9)

Immunoglobulin classification showed the ex pected distribution in uncompliated myeloma with marked dominance of IgG about 10% of IgA myeloma and one case (5%) of light chain disease As expected there was kappa chain predomination in the M-components of this group of patients (75%) We therefore conclude that no particular group with respect to M component class or group could be identified among patients with myeloma and paraparesis

A study of laboratory variables such as Hb serum calcium serum alkaline phosphatase and ESR in the 20 patients did not disclose any particu lar pattern to distinguish them from patients with uncomplicated myelomatosis nor did the semi quantitative estimation of urinary monoclonal light chains differ Renal failure was apparently not pres ent more commonly in the paraparesis group than in myeloma patients without paraparesis

There was marked localization of myelomatous lesions to the thoracic spine causing paraparesis (Fig 3) This is probably explained by the fact that vertebral body compression in the lumbar spine cannot cause medullary damage since the spinal medulla does not usually extend beyond vertebra Th 12 Damage to the cervical spine could cause paraparesis or tetraplegia depending on the level of the lesion We cannot explain the lack of involve ment of the cervical spine in our patients

Of the 20 patients 16 had paresis of the blad der causing incontinence of urine A marked sensory loss in the lower extremities both objective and subjective was also found in 17 patients

It is clear that it is worthwhile to diagnose and treat this fairly common complication of my elomatosis At the time of writing 8 patients of 20 are still alive the mean survival after operation being 38 months (range 3–136) The mean post operative survival of the 12 patients who died was 12 months (range 0–52) Functionally the results of treatment are encouraging 6 of 19 operated patients showing full recovery and 6 a partial recovery Eight patients remained paraparetic after operation irradiation and cytostatic therapy probably owing to excessive duration of medullary compression (Fig 2)

Considering the serious consequences of medul lary damage secondary to myelomatous collapse of vertebral bodies in the thoracic spine perhaps it might be expedient to give prophylacitc irradiation to all lesions in the thoracic spine detected by X ray or radionuclide scan

REFERENCES

1 Alberts D S Dune B G M & Salmon S E Doxorubicin/B C N U chemotherapy for multiple myeloma in relapse Lancet 1 926 1976

2 Alexanian R Haut A Khan A U Lane M McKelvey E M Migliore P J Stuckey W J & Wilson H Treatment for multiple myeloma Combi nation chemotherapy with different melphalan dose regimens JAMA 208 1680 1969

3 Bergsagel D E Cowan D H & Hasselback R Plasma cell myeloma response of melphalanresistant patients to high-dose intermittent cyclophosphamide Can Med Assoc J 107 851 1972

4 Cohen H J & Rundles R W Managing the com plications of plasma cell myeloma Arch Intern Med 135 177 1975

5 Garrett M J Spinal myeloma and cord compres-sion—diagnosis and management Clin Radiol 20 42 1969

6 Hiller A Greif R L & Beckman W W Determi nation of protein in urine by the biuret method J Biol Chem 176 1421 1948

7 Hindmarsh T Myelography with the non ionic water soluble contrast medium metrizamide Acta Radiol 16 417 1975

8 Hoogstraten B Costa G Cuttner J Forcier J Leone L A Harley J B & Glidewell O J In termittent melphalan therapy in multiple myeloma JAMA 209 251 1969

9 Hubner K F Gould A A Hayes R L Pog genburg J K & Solomon A The use of rare-earth radionuclides and other bone seekers in the evalua tion of bone lesions in patients with multiple myeloma or solitary plasmacytoma Radiology 125 171 1977

10 Johansson B G Agarose gel electrophoresis Scand J Clin Lab Invest (Suppl) 124 7 1972

11 Lindstedt G & Lundberg P A Loss of tubular *proteinuria pattern during urine concentration with a* commercial membrane filter cell (Minicon B 15 system) Clin Chim Acta 56 125 1974

12 Lindstrom F D & Dahlstrom U Multiple myeloma or benign monoclonal gammapathy (BMG)? A study of differential diagnostic criteria in 44 cases of BMG Clin Immunol Immunopathol 10 168 1978

13 Lizana J & Hellsing K Polymer enhancement of automated immunological nephelometric analysis as illustrated by determination of urinary albumin Clin Chem 20 415 1974

14 Mancini G Carbonara A D & Heremans J F Immunochemical quantitation of antigens by single radial immunodiffusion Immunochemistry 2 235 1965

15 McArthur J R Athens J W Wintrobe M A & *Cartwright G E Melphalan and myeloma* Experience with a low-dose continuous regimen Ann Intern Med 72 665 1970

16 Silverstein A & Doniger D E Neurologic complications of myelomatosis Arch Neurol 9 534 1963

17 Snapper I & Kahn A Myelomatosis University Park Press Baltimore 1971

18 Stavem P Froland S S Haugen H F & Lislerud A Non secretory myelomatosis without intracellular immunoglobulin Scand J Haematol 17 89 1976

19 Williams R C Brunning R D & Wollheim F A Light chain disease—an abortive variant of multiple myeloma Ann Intern Med 65 471 1966

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Comparison of Biopsy Procedures in Intrathoracic Sarcoidosis

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ABSTRACT A series of 78 patients, mainly young males with intrathoracic manifestations of sarcoidosis, found on routine chest X ray, were examined according to a prescheduled program including different biopsy procedures In 76% the intrathoracic changes were isolated hilar adenopathy in 9% isolated pulmonary lesions and in 15% hilar adenopathy plus pulmonary lesions Lymph nodes containing granulomas were found by mediastinoscopy in 41 of 44 patients and by scalene fat pad biopsy in 20 of 34 patients In 27 of 76 patients granulomas were found by liver biopsy Complications to the biopsies were more frequent with mediastinoscopy than with the other two biopsy procedures but no serious complications occurred Biopsy through mediastinoscopy is thus preferable in patients of this kind The necessity of doing biopsies to support the diagnosis of sarcoidosis is discussed on the basis of the literature and our own studies In younger patients with asymptomatic bilateral hilar adenopathy without pulmonary lesions it seems justifiable to omit biopsy whereas biopsy is mandatory in patients with unilateral hilar lymph adenopathy and patients with pulmonary lesions In all cases the course of the disease should be followed for a rather long period

Key words Sarcoidosis bilateral hilar adenopathy mediastinoscopy scalene fat pad biopsy liver biopsy tuberculin reaction

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Various biopsy procedures (15 19 24 29 33 34) have been used in studies of patients with intrathoracic manifestations of sarcoidosis in order to support the diagnosis histologically It has been discussed in these studies which biopsy procedure is preferable and whether it is necessary to perform a biopsy in every case (22 23 30 38)

In a previous study (24) of patients with intrathoracic radiographic changes of suspected sarcoid origin we found granulomas in about 60% through scalene fat pad biopsy and in about 40% by liver biopsy In the present study of a similar group of patients we have compared the results of biopsy by mediastinoscopy scalene fat pad biospy and liver biopsy to illustrate problems concerning the choice of biopsy procedure and the indications for doing biopsies in patients of this kind

PATIENTS

During the period 1968–75 80 patients were admitted to Medical Department TA Rigshospitalet Copenhagen because routine chest X ray had demonstrated hilar lymph adenopathy and/or pulmonary infiltrates suspected of being of sarcoid origin

During the admission 2 patients received the diagnosis of Mb Hodgkin without histological changes in lymph node biopsies compatible with sarcoidosis Seventy three of the 78 patients with suspected sarcoidosis 74 males and 4 females were between 16 and 30 years 5 between 31 and 50 years of age Sixty five were young draftees 7 belonged to other categories of military personnel and 6 were civilians None of the patients was known to suffer from any other major disease Interrogation and examination upon admission revealed minor complaints and/or physical signs in 24 of the 78 patients sneezing and coughing in 15 joint and muscle pains in 4 slightly enlarged peripheral lymph nodes in 9 erythema nodosum in 6 slightly elevated temperature in 3

METHODS

All the 78 patients went through a prescheduled examination program which included different biopsies Altogether 159 biopsies two or more in 74 patients were performed (Table I) During the first 2 years of the 7 year period the examination program included scalene

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Abbreviations BHL=bilateral hilar adenopathy T tuberculin unit

Table I *Results of different biopsy procedures in 78 patients*

D=detected ND=not detected

	No of biopsies	Suitable tissue obtained	Granulomas	
			D	ND
Mediastinoscopy	44	41	41	
Scalene fat pad biopsy	34	31	20	11
Thoracotomy	2	2	2	
Peripheral lymph node biopsy	2	2	1	1
Liver biopsy	76	75	27	48
Skin–muscle biopsy	1	1		1
Total	159	152	91	61

fat pad biopsy This biopsy was performed in 34 patients as described by Daniels (7) in Department K for head and neck surgery under general anesthesia in 32 cases and local anesthesia in 2 In the last 5 years of the 7 year period lymph node biopsy was done by mediastinoscopy if the patient had hilar lymph adenopathy This biopsy procedure was undertaken in 44 of the patients in Department R for thoracic surgery using the technique described by Carlens (4) Liver biopsy a m Menghini (20) was performed in 76 of the patients All biopsy specimens were examined and described by the Department of Pathology

Routine blood analyses (including serum creatinine serum calcium and liver function tests) were carried out in all patients Serum electrophoresis was performed in patients IgG IgA and IgM were determined in 44 ents The toxoplasmosis neutralization test and the plement fixation test were made in 66 patients Mantoux reaction with 1 TU (tuberculin unit) and/or 10 TU (PPD State Serum Institute Copenhagen) was tested in 67 patients

After discharge all the patients were referred to follow up chest X ray within one year either at our department or local chest clinics

The computer assisted statistical analysis of data was performed by J Nyboe Statistical Department University Hospital Rigshospitalet Copenhagen

RESULTS

Radiographic intrathoracic manifestations

Fifty nine patients (76%) had isolated hilar lymph adenopathy 7 (9%) isolated pulmonary lesions and 12 (15%) both hilar lymph adenopathy and pulmonary lesions *Hilar lymph adenopathy* was bilateral and symmetrical (BHL) in 68 and unilateral in 3 patients The pulmonary lesions consisted of isolated infiltrates in one or both lungs in 12 patients and in widespread mottled shadowing in 7

Mediastinoscopy

A lymph node was obtained in 41 of the 44 patients who underwent mediastinoscopy It contained typical epithelioid cell granulomas in all 41 cases (Table I)

Two of the 3 patients in whom no lymph node was obtained had unilateral hilar lymph adenopathy In one of these 3 patients it was impossible to introduce the mediastinoscope because of a firm resistance of unknown origin Tissue suitable for histological examination was obtained by scalene fat pad biopsy but it did not contain epithelioid cell granulomas A subsequent thoracotomy yielded a lymph node containing granulomas In another of these 3 patients mediastinoscopy could not be carried out because of abnormal vascular structures and bleeding—although not serious Immediately after the attempted mediastinoscopy a scalene fat pad biopsy was done and tissue containing granulomas was obtained In the third patient mediastinoscopy was likewise abandoned because of abnormal vascular structures Thoracotomy was therefore performed and a lymph node with granulomas was found

Scalene fat pad biopsy

This biopsy was done in 34 patients and lymph node tissue was obtained from 31 of them In 20 cases the tissue contained epithelioid cell granulomas (Table I)

Liver biopsy

Liver tissue was obtained from 75 of the 76 patients in whom liver biopsy was performed In 27 cases the liver tissue contained granulomas (Table I)

Other biopsies

In 5 patients other biopsy procedures were used Two of them had a lymph node removed through thoracotomy as described above Two patients had a peripheral palpable lymph node removed and in one patient a skin–muscle biopsy was done In all 5 cases tissue suitable for histological examination was obtained and granulomas were present in 3 cases Altogether granulomas were found in 91 tissue biopsies from 68 patients (Table I)

Comparison of biopsies

In Table II the histopathological findings in biopsy material from 38 patients obtained through

Table II *Tissue suitable for histological examination obtained by liver biopsy and mediastinoscopy (38 patients) or by liver biopsy and scalene fat pad biopsy (31 patients)*

	Liver biopsy		
	Granulomas detected	Granulomas not detected	No of pats
Mediastinoscopy			
Granulomas detected	13	25	38
Granulomas not detected	0	0	0
Total	13	25	38
Scalene fat pad biopsy			
Granulomas detected	10	10	20
Granulomas not detected	2	9	11
Total	12	19	31

mediastinoscopy are compared with those obtained by liver biopsy from the same 38 patients As seen granulomas were found by mediastinoscopy in all cases but in only 13 by liver biopsy The results in 31 patients who underwent both scalene fat pad biopsy and liver biopsy are also shown in Table II Granulomas were found in 20 of the 31 patients by scalene fat pad biopsy and in 12 by liver biopsy In 10 patients granulomas were found by both kinds of biopsy and in 9 patients by neither of the biopsies Thus granulomas were found more frequently by scalene fat pad biopsy than by liver biopsy

Complications

Nineteen of the 44 patients who underwent mediastinoscopy had slight and transient complications Fifteen patients had elevated temperature to about 38 C two to a maximum of 39 9°C which normalized within 1–5 days Five patients had transitory pains in the chest two complained of hoarseness and one had a short lasting bleeding Serious complications were not seen Six out of 34 patients subjected to scalene fat pad biopsy had slightly elevated temperature for periods of less than 36 hours No other complications were seen Nine of the 76 patients who underwent a liver biopsy had slight complications such as transient hypotension pains in the right shoulder and slight abdominal pains None had serious complications

Other examinations

No patients had signs of liver disease or enlargement of the liver In the cases with abnormal liver function tests the deviations from normal were minor Serum gammaglobulin concentration was elevated in 5 (11 6–14 4 g/l) out of 30 patients examined IgG was elevated in 7 (16 2–35 6 g/l) IgA in 3 (3 38–4 20 g/l) and IgM in one (1 36 g/l) of 44 patients examined Elevated IgG was found more often in patients with than without granulomas present in the liver biopsy (4 of 7)

Serum creatinine was normal in the 78 patients examined and serum calcium concentration was elevated in one Among 68 patients examined ESR was elevated in 14 (11–103 mm/hour)

The toxoplasmosis neutralization test was positive in 16 of 66 patients The titers were 1/10 in 9 patients and ranged in 7 from 1/50 to 1/250 The toxoplasmosis complement fixation reaction was positive in 13 of 66 patients The titers were 1/2 or less in 11 patients and 1/8 and 1/16 respectively in 2 patients

Table III shows that 63% (of 67 patients tested) had a positive intracutaneous tuberculin reaction (≥10 mm) with 1 or 10 TU

DISCUSSION

The ratio between patients with BHL and patients with isolated pulmonary lesions is almost the same in this as in our previous study (24) the majority of patients having BHL as the only manifestation This is to be expected since age and selection of the

Table III *Intracutaneous tuberculin reaction in 67 patients*

D=detected ND=not detected Positive reaction ≥10 mm

	No of pats		Granulomas	
			D	ND
Positive with 1 TU	27	[illegible]	[illegible]	[illegible]
Positive with 10 TU	15			[illegible]
Negative with 1 or 10 TU	25			
Total	6[illegible]			

patients in the two series are almost identical In accordance with this we also found almost the same percentage of granulomas by scalene fat pad biopsy in the two studies 62 and 65% These percentages are in accordance with or a little lower than the percentages of positive biopsies found in other investigations (14 29 30 34)

However in the present study lymph node biopsy was performed through mediastinoscopy in a majority of the patients Granulomas were detected in all 41 patients in whom a lymph node was obtained by mediastinoscopy The examination was unsuccessful in 3 patients as described above Thus our results are in agreement with those of others (3 5 16 21 27 29) who have found granulomas in 80–100 % of patients with BHL subjected to mediastinoscopy We have not attempted mediastinoscopy in any of the 7 patients who had pulmonary lesions without radiographically demonstrable enlargement of the hilar lymph nodes

In a similar group of patients with isolated pulmonary lesions without BHL granulomas were found in 2 of 4 patients by mediastinoscopy (36) and in 8 of 8 patients by transbronchial lung biopsy (18) We found granulomas by scalene fat pad biopsy and/or liver biopsy in 5 of 7 patients with isolated pulmonary lesions without BHL In the last two of these patients granulomas were not detected by either of these two biopsy procedures

In the present study granulomas were found by er biopsy in 36% of the cases in accordance with r previous results This is definitely a lower percentage than what is reported in most other studies (14 17 19 34)

One explanation may be differences in the selection of patients almost all our patients being young apparently healthy persons Another explanation could be differences in the size of the biopsy and in the histological technique One of the investigators above (17) found an especially high percentage (94%) of granulomas by liver biopsy He relates this to the fact that he takes very large biopsies and examines them by many serial cuts We have not made any special efforts to obtain large biopsy specimens most of them were less than 20 mm long but a large number of cuts have been performed routinely Lehmuskallio et al (21) who also found a rather low incidence of positive liver biopsies mention the possibility of a more benign course of sarcoidosis in the northern European countries Liver function tests and other laboratory tests were normal in most patients in the present study

The toxoplasmosis neutralization test and the toxoplasmosis complement fixation test were positive in 16 and 13 respectively out of 66 patients examined However in most cases the titers were low The significance of positive serological reactions for toxoplasmosis in patients with sarcoidosis cannot be estimated until an examination of a similar group of the Danish population without sarcoidosis is available

A positive intracutaneous tuberculin test was found in 63% of 67 patients This is a higher percentage than found by most other investigators especially compared to most results from the USA The reason for a higher incidence of positive tuberculin reaction in northern Europe than for instance in the USA has been discussed It has been suggested that it might be due to the widespread use of BCG in northern Europe (6) A positive tuberculin test was found in 55% of 150 patients from Finland (31) just as we in our previous study (24) found a positive tuberculin test in 59% of 1?9 patients

Our results show that in one and the same group of patients tissue specimens containing granulomas are found more often by mediastinoscopy than by liver biopsy This is to be expected because mediastinoscopy unlike biopsy of the liver permits removal of lymphoid tissue from a region where the disease in most cases is first recognized It has been claimed that biopsy of the liver through laparoscopy reveals granulomas nearly as frequently as biopsy by mediastinoscopy (35) However this probably implies visible changes on the surface of the organ Fotí and Moser (10) found granulomas by scalene fat pad biopsy as frequently as by liver biopsy but they compared the two biopsy procedures in a rather small number of patients In our present and previous study (24) we found granulomas more often by scalene fat pad biopsy than by liver biopsy in a large group of patients with intrathoracic radiographic changes suspected to be sarcoidosis This finding is in accordance with the fact that granulomas are detected by lymph node biopsy through mediastinoscopy in almost all patients with BHL We have not performed concurrent mediastinoscopy and scalene fat pad biopsy in one and the same patient group to our knowledge only one study (9) compares the two biopsy methods in that way in an effort to

evaluate their efficacy in the diagnosis of intra thoracic lesions Of the 15 patients in whom granulomas were found by mediastinoscopy only 11 exhibited granulomas by scalene fat pad biopsy These results as well as ours make it probable that granulomas are found more frequently by mediastinoscopy than by scalene fat pad biopsy

When evaluating biopsy procedures however the frequency of complications has also to be taken into consideration Serious complications to me diastinoscopy are unusual in extensive materials (4 16 28 37) but 4 fatal incidents have been report ed in connection with this procedure (1, 32) In all 4 cases the patients suffered from malignant diseases and it is dubious whether the deaths could be ascribed to the biopsy procedure In patients with sarcoidosis no fatal incidents have been reported in association with mediastinoscopy but a few cases *with severe hemorrhage (3 25 38) and one case* with lesion of the esophagus (26) One of our pa tients developed a moderate hemorrhage which was stopped by simple tamponade However the proce dure was given up in this patient and in two others because of abnormal vascular structures This is known to increase the risk of the examination (16) Fifteen of our patients had elevated temperature for a few days as mentioned Otherwise no complica tions related to mediastinoscopy occurred in our comparatively unaffected young patients This confirms that mediastinoscopy is a reliable method in diagnosing sarcoidosis usually with no or slight complications The drawbacks of the method are that it needs a specially trained surgeon and that it can induce mediastinal fibrosis which may impede another mediastinoscopy in one and the same pa tient (16 28) The complications to scalene fat pad biopsy and liver biopsy were few and not serious

In conclusion biopsy by mediastinoscopy is the method to be preferred in patients with hilar lymph adenopathy because of its diagnostic certainty This certainty probably arises from the fact that an ade quate and representative tissue specimen can be obtained in the majority of cases by this biopsy procedure However complications are more fre quent and serious by mediastinoscopy than by the other two biopsy procedures (scalene fat pad biopsy and liver biopsy) used in this study Scalene fat pad biopsy is still of value in confirming the diagnosis of sarcoidosis in some cases and has the advantage of being repeatable at later occasions

Granulomas were demonstrated in 35–40% by liver biopsy in our studies but as mentioned many authors have obtained higher percentages

However one must also consider whether it is always necessary to confirm the diagnosis of sarcoidosis by biopsy from one or more organs Winterbauer et al (38) on the basis of a study including 99 patients with sarcoidosis expressed the opinion that biopsy is not necessary in patients with asymptomatic symmetrical BHL and in pa tients with BHL plus uveitis and/or erythema nodosum because the findings are so typical for sarcoidosis Some authors agree with (13 22 30) and others are opposed to this opinion (8 11) but there is no convincing information against the statement of Winterbauer et al Our results support their point of view especially concerning the fact that all the 41 patients in whom granulomas were demonstrated by biopsy through mediastinoscopy *had asymptomatic symmetrical BHL*

One of our 3 patients with unilateral hilar adenopathy developed Mb Hodgkin later in the course Coincidence of sarcoidosis and Mb Hodgkin in one and the same patient has been described before (2 23 30) Furthermore it should be mentioned that the two patients who had Mb Hodgkin but not sarcoidosis both had unilateral hilar adenopathy These patients stress the necessity of some kind of biopsy in patients with unilateral hilar adenopathy while we consider it justifiable to omit biopsy in young patients with asymptomatic bilateral hilar lymph adenopathy This attitude also takes into consideration the potential risk of biopsies in this kind of young clinically healthy persons usually with a good prognosis (12 30 38) However the diagnostic value of following the course of the dis ease in the individual patient for a rather long period must be stressed This applies both to patients in whom granulomas have or have not been demon strated

ADDENDUM

After this manuscript has been submitted for publication we have become aware of a fatal incidence during medi astinoscopy in a patient included in a study of the vital prognosis of intrathoracic sarcoidosis (Viskum K & Thygesen K Scand J respir Dis 53 181 1972

REFERENCES

1 Bergh N P Rydberg B & Schersten T Dis Chest 46 399 1964
2 Brincker H & Wilbek E Br J Cancer

patients in the two series are almost identical In accordance with this we also found almost the same percentage of granulomas by scalene fat pad biopsy in the two studies 62 and 65% These percentages are in accordance with or a little lower than the percentages of positive biopsies found in other investigations (14 29 30 34)

However in the present study lymph node biopsy was performed through mediastinoscopy in a majority of the patients Granulomas were detected in all 41 patients in whom a lymph node was obtained by mediastinoscopy The examination was unsuccessful in 3 patients as described above Thus our results are in agreement with those of others (3 5 16 21 27 29) who have found granulomas in 80–100 % of patients with BHL subjected to mediastinoscopy We have not attempted mediastinoscopy in any of the 7 patients who had pulmonary lesions without radiographically demonstrable enlargement of the hilar lymph nodes

In a similar group of patients with isolated pulmonary lesions without BHL granulomas were found in 2 of 4 patients by mediastinoscopy (36) and in 8 of 8 patients by transbronchial lung biopsy (18) We found granulomas by scalene fat pad biopsy and/or liver biopsy in 5 of 7 patients with isolated pulmonary lesions without BHL In the last two of these patients granulomas were not detected by either of these two biopsy procedures

In the present study granulomas were found by er biopsy in 36% of the cases in accordance with r previous results This is definitely a lower percentage than what is reported in most other studies (14 17 19 34)

One explanation may be differences in the selection of patients almost all our patients being young apparently healthy persons Another explanation could be differences in the size of the biopsy and in the histological technique One of the investigators above (17) found an especially high percentage (94%) of granulomas by liver biopsy He relates this to the fact that he takes very large biopsies and examines them by many serial cuts We have not made any special efforts to obtain large biopsy specimens most of them were less than 20 mm long but a large number of cuts have been performed routinely Lehmuskallio et al (21) who also found a rather low incidence of positive liver biopsies mention the possibility of a more benign course of sarcoidosis in the northern European countries. Liver function tests and other laboratory tests were normal in most patients in the present study

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Non-Invasive Beat-to-Beat Analysis of Stroke Volume and Digital Pulse Volume in Patients with Complete Heart Block and Artificial Pacing

B Fagrell[1] and K Lindvall

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ABSTRACT This study presents a beat to beat analysis of digital arterial pulse volume (DAPA), left ventricular end diastolic diameter (LVEDD), and stroke volume (SV) and their correlation to PQ interval in ten patients with complete heart block and artificial cardiac pacing DAPA was measured by *strain gauge plethysmography* and LVEDD/SV by echocardiography A close relationship was found between SV and DAPA (r=0 83–0 97) in seven patients who all drew considerable benefit from atrial contraction as regards SV and DAPA (increase with 35–94%) The optimal PQ interval was calculated to approximately 240 msec for DAPA and 180 msec for *LVEDD and SV It may be concluded that the present* study demonstrates a close relationship between beat to beat variations of SV measured by echocardiography and plethysmographically recorded digital arterial pulse volume These variables may be useful in clinical practice for assessing the hemodynamic effect of atrial contribution in patients with various forms of cardiac conduction disturbances The two methods may for instance be useful for screening in order to pick out patients who may benefit from AV synchronous rather than ventricular pacing

Key words artificial pacing stroke volume echocardiography digital pulse volume pulse plethysmography

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Most patients treated with artificial pacemakers for severe cardiac conduction disturbances obtain great benefit from this treatment However in some instances artificial pacing may have deleterious effects on both central and peripheral circulation (2) In these atrioventricular (AV) dissociation may cause intermittent valvular incompetence when atrial systole occurs at a certain point during the ventricular contraction (8) Interference between the patient s own conducting system and the artificial pacemaker activity is another reason for a possible negative hemodynamic effect

So far in order to evaluate such disturbances cardiac catheterization has been performed However reliable non invasive techniques would be more suitable for clinical practice Echocardiography is one way of studying central hemodynamics non invasively (4 5 7 12 17) and the method is particularly well suited to measure beat to-beat stroke volume (SV) variations (14) Marked changes in the central circulation often cause simultaneous changes in the peripheral circulation Consequently certain variables of the peripheral circulation may be used to reflect changes in the central hemodynamics Accordingly peripheral pulse volume analysis has been shown to be of value for assessing hemodynamic consequences of artificial pacing in man (3)

In the present report beat to-beat SV measured by echocardiography has been correlated to the finger pulse volume in patients with persistent sinus activity and complete heart block and various circulatory disorders e g dizziness and palpitations The purpose of the study was 1) To determine beat to-beat variations of left ventricular end-diastolic diameter (LVEDD) and SV measured by echocardiography and digital arterial pulse volume (DAPA) recorded plethysmographically 2) to calculate intercorrelations between LVEDD/SV and DAPA 3) to evaluate the importance of the timing of the arterial activity for the above mentioned variables

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Abbreviations BBA=beat to-beat analysis DAPA=digital arterial pulse volume LVEDD=left ventricular end diastolic diameter SV=stroke volume AV=atrioventricular SA=sinoatrial

Table I *Type of underlying disease conduction disturbance and AV rate during investigation*
IHD = ischemic heart disease CB = congenital AV block CU = cause unknown CP = cor pulmonale

Pat no	Age (y)	Sex	Underlying disease	Conduction disturbance	Rate of Ventricle	Rate of Atrium
1	63	♂	IHD	AV III	70	66
2	59	♂	IHD	AV III	70	75
3	58	♀	IHD	AV III	66	74
4	57	♂	CP	SA interm	60	64
5	47	♀	CU	AV III	36*	76
6	75	♀	IHD	AV III	70	64
7	25	♀	CB	AV III	52*	85
8	85	♀	IHD	AV II–III	45*	85
9	79	♂	IHD	AV III	70	85
10	62	♀	IHD	AV III	73	90

* Patients without pacemaker at the time of investigation

PATIENTS AND METHODS

Studies were made on ten patients aged 25–85 years with complete heart block or intermittent sinoatrial (SA) block (Table I) Seven were treated with artificial pacemaker Six patients were women (three treated) and four men (all treated) The cause of the conduction disturbance was ischemic heart disease in seven cor pulmonale in one congenital heart disease in one and unknown in one All patients were studied after 10 min rest in supine or in 30° right anterior oblique position with the trunk raised about 30°

Echocardiography An Organon Teknika Echocardiovisor (2 25 mHz transducer focused on 7 5 cm) was used and the findings were displayed on a Honeywell line scan recorder at a rate of 25 or 50 mm/sec simultaneous recordings of ECG and digital hysmography (Fig 1) The transducer was placed parasternally over the third or fourth intercostal space The registration was accepted when the left posterior wall was identified behind the posterior mitral valve simultaneously with the left ventricular septal margin The diastolic diameter was calculated after atrial contraction had occurred at a maximally dilated left ventricle which in patients with pacing occurs at the end of the R wave of the QRS complex The systolic diameter was measured when the posterior wall was closest to the septum (4 12) Diastolic and systolic volumes were calculated according to the technique of Teicholz et al (17)

Digital strain gauge plethysmography The investigation was performed according to the technique described by Hallbook et al (9) The strain gauge was applied around the distal phalanx of the left thumb The pulse volume curve was amplified until the recorded amplitude was between 10 and 20 mm Changes in pulse amplitude were used as an index of digital pulse volume variations (9)

Analysis of data Fifteen consecutive beats were used for the analysis

1) LVEDD (mm) and SV (ml) measured by echocardiography were correlated to DAPA (mm) measured plethysmographically 2) LVEDD as well as SV and DAPA for each pulse cycle were plotted against the PQ interval Different values of LVEDD SV and DAPA were calculated at zero PQ time and also the PQ time at the maximal values of LVEDD SV and DAPA

RESULTS

Correlation between LVEDD/SV and DAPA The data are shown in Fig 2 and Table II Beat to-beat analysis (BBA) of the relationship between LVEDD/SV and DAPA showed a significant correlation ($r=0.53$–0.97 $p<0.05$–0.001) in seven patients but no significant correlation ($r=0.18$–0.02 $p>0.05$) in three (Fig 2) In two of the latter pa

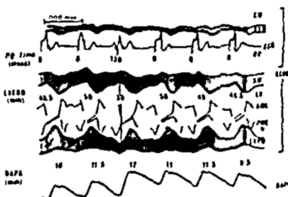


Fig 1 Simultaneous registration of echocardiogram (*ECHO*) elektrocardiogram (*ECG*) and digital arterial pulse amplitude (*DAPA*) in a patient with intermittent SA block and artificial pacemaker activity Paper speed 25 mm/sec *CW* = chest wall *RVW* = right ventricular wall *SW* = septal wall *LV* = left ventricular *AML* and *PML* = anterior resp post mitral leaflet *LVPW* = left ventricular posterior wall

Table II *LVEDD (mm) SV (ml) and DAPA (mm) at zero and optimal PQ times (msec) and increases in the recorded variables*

Pat no	DAPA at PQ time 0	Max DAPA at optimal PQ time*	Increase in DAPA (%)	LVEDD/SV at PQ time 0	Max LVEDD/SV at optimal PQ time*	Increase in LVEDD/SV (%)
1	13	18 (140)	38	39/36	43/48 (140)	10/33
2	14 5	22 5 (180)	55	51/52	55/79 (200)	10/49
3	10	17 (240)	70	34 5/17	38 5/33 (130)	11/94
4	9 5	17 (160)	79	49 5/70	59/128 (160)	19/82
5	17	19 (410)	12	64/121	66/135 (320)	3/11
6	10	15 (160)	50	42/46	50/71 (160)	19/54
7	11 5	14 (320)	21	52/90	55/108 (270)	6/20
8	8	13 (300)	62	52/85	63/156 (220)	17/83
9	23	31 (150)	35	66/136	74/203 (160)	16/49
10	10	18 (340)	80	41/49	51/92 (40)	18/87
Mean	12 7	18 5 (240)	50	49 1/70 3	55 5/105 3 (180)	13/56
± S D	4 5	5 2 (96)	23	10 3/37 8	10 6/51 7 (78)	6/29

*Optimal PQ time given in parentheses

tients (nos 5 and 7) very small variations in LVEDD/SV and DAPA were recorded In a third patient (no 3) an intermittent mitral insufficiency was recorded phonographically when the atrial contractions appeared approximately 50 msec after the onset of the QRS complex Consequently more prominent variations were recorded in DAPA than in LVEDD and SV

Correlation between PQ interval and LVEDD SV and DAPA respectively The data are shown in

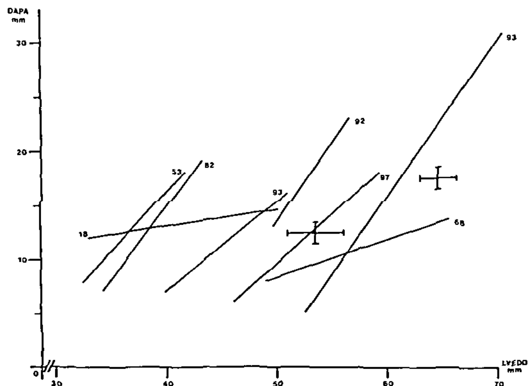


Fig 2 Syncronous beat to-beat analysis of LVEDD and DAPA The regression coefficient for each line is given

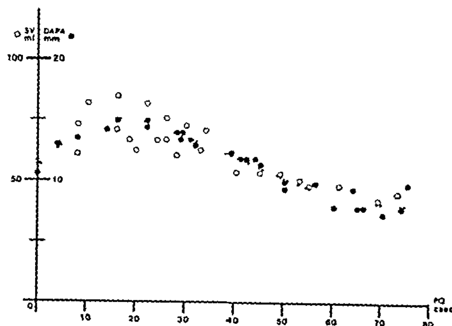


Fig 3 Effect of various PQ intervals on SV and DAPA in patient 6 Note the close correlations

Fig 3 and Table II The mean DAPA at zero PQ time was 12 7±4 5 (mean ± S D) mm and increased to 18 5±5 2 mm at the optimal PQ time of 240±96 msec The mean increase in DAPA was 50±23% when ventricular contractions were preceded by atrial activity The values of LVEDD and SV at zero PQ time were 49 1±10 3 mm and 70 3±37 8 ml respectively LVEDD and SV increased to 55 5±10 6 mm and 105 3±51 7 ml respectively at the optimal PQ time of 180±78 msec This corresponds to an increase in LVEDD and SV of 13±6% and 56±29% respectively A significant correlation ($p<0.01$) was found between the percentage increases in LVEDD and SV compared to the increase in DAPA (Fig 4)

DISCUSSION

In recent years several reports have convincingly shown that left ventricular volumes can be obtained

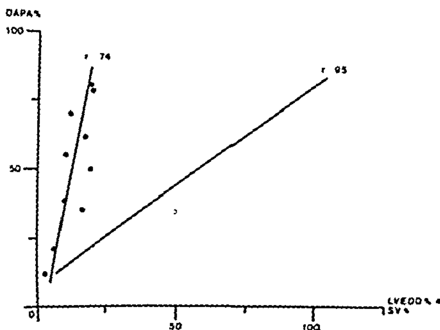


Fig 4 Increases in LVEDD SV and DAPA at ventricular contractions without preceding atrial activity (PQ=0 msec) to the maximal value at optimal PQ time A very good correlation (p 0 01) found between LVEDD SV and DAPA

accurately with echocardiography (4 5 7 12 17) Comparative studies between cineangiography and echocardiography for measuring left ventricular volumes have shown strong correlations (r= 0 85–0 95) However in certain groups of patients e g patients with coronary heart disease or other causes of left ventricular asynergy this relationship is less valid (10 11 17) The error of repetitive measurements made by the same or different investigators is rather small (6 7 11 14) though inaccurate angling of the transducer will lead to errors (16)

Only relative changes from one beat to another of LVEDD SV and DAPA have been used in the present study The error of intraindividual LVEDD and SV measurements by echocardiography will be constant in BBA and may consequently be neglected (14) In seven out of ten patients the correlations between LVEDD SV and DAPA were highly significant ($p<0\ 01$–$0\ 001$) and significant in one ($p<0\ 05$) In the latter patient auscultation and phonocardiography revealed intermittent mitral insufficiency which resulted in a more marked drop in DAPA than in SV in beats in which regurgitation from the left ventricle was present In two patients minimal variations were found for LVEDD SV and DAPA suggesting that atrial activity had but a minor effect on SV and DAPA In the other patients atrial activity contributed to an increase in SV and DAPA by 35–94%

BBA of DAPA and SV as well as LVEDD permitted the determination of optimal PQ intervals The close correlation between DAPA and SV shows that changes in SV are reflected by changes in DAPA These results confirm previous findings obtained by cardiac catheterization where proper timing of atrial contractions in most cases significantly improved cardiac function in patients with e g AV block (3 8) Brand et al (1) who studied the hemodynamic effects of AV sequential pacing in five patients with complete heart block found different responses in effective cardiac outputs In three patients SV was higher in one equal and in one lower during AV sequential than during fixed rate pacing This finding also indicates that in some patients properly timed atrial activity does not seem to improve cardiac output Our two patients who failed to improve SV were the youngest and their myocardium can be assumed to be undamaged This agrees with the finding of Rahimtoola et al (13) who showed that atrial contribution to ventricular filling is significantly less in normal subjects than in patients with myocardial infarction and largest in patients with low cardiac output

BBA of the effect of atrial activity on ventricular performance has been performed in only a few studies to date Sapoznikov et al (15) measured left ventricular preejection period and pulse wave velocity during complete heart block and artificial pacing They showed by BBA that the femoral and dorsalis pedis pulses significantly changed in amplitude during various types of pacing In another report (3) they also assessed the optimal PQ interval for atrial contribution to ventricular performance by using dorsalis pedis pulse volume analysis The optimal PQ time for the arterial pulse volume was inversely related to ventricular rates and varied from 180 to 360 msec They conclude that the relative inefficiency of beats preceded by very short or very long PQ intervals may be of clinical significance in situations like nodal rhythm or AV blocks This agrees with the findings in our patients treated with artificial pacemaker

It may be concluded that the present study demonstrates a close relationship between BBA of SV measured by echocardiography and plethysmographically recorded DAPA There are large variations in atrial contribution to SV from one patient to another DAPA analysis and SV measurement by echocardiography may be useful in clinical practice for assessing the hemodynamic effect of atrial contribution in patients with various forms of cardiac conduction disturbances These methods may be used for screening in order to pick out patients who may benefit from AV synchronous rather than ventricular pacemakers

ACKNOWLEDGEMENT

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REFERENCES

1 Brand M V D Berkovits B V Hugenholtz P G & Meester G T Left ventricular function during A–V sequential pacing in total A–V block In Cardiac pacing Proceedings of the IVth International Symposium on Cardiac Pacing p 133 Groningen The Netherlands 1973
2 Edhag O Fagrell B & Lagergren H Deleterious effects of cardiac pacing in a patient with mitral insufficiency Acta Med Scand 202 331 1977
3 Eliakim M Sapoznikov D & Weinman J Assess

ment of the atrial contribution to cardiac performance by a non invasive photoplethysmographic technique Cardiology 58 7 1973

4 Feigenbaum H Clinical applications of echocardiography Prog Cardiovasc Dis 14 531 1972

5 Fortuin N J Hood W P Sherman M E & Craige E Determination of left ventricular volumes by ultrasound Circulation XLIV 575 1971

6 Gerstenblith G Fredriksen J Yin F C P Fortuin N J Lakatta E G & Weisfeldt M L Echocardiographic assessment of a normal aging population Circulation 56 273 1977

7 Gibson D G Estimation of left ventricular size by echocardiography Br Heart J 35 128 1973

8 Haas J M & Strait G B Pacemaker induced cardiovascular failure Hemodynamic and angiographic observation Am J Cardiol 33 295 1975

9 Hallbook T Månsson B & Nilsén R A strain gauge plethysmograph with electrical calibration Scand J Clin Lab Invest 25 413 1970

10 Ludbrook P Larliner J S Peterson K Leopold G & O Rourke R A Comparison of ultrasound and cineangiographic measurement of left ventricular performance in patients with and without wall motion abnormalities Br Heart J 35 1026 1973

11 Pombo J F Russel R O Rackley C E & Foster G L Comparison of stroke volume and cardiac output determination by ultrasound and dye dilution in acute myocardial infarction Am J Cardiol [illegible] 630 1971

12 Popp R L & Harrison D C Cardiac chamber size and volume In Echocardiography Year Book Medical Publishers Chicago 1974

13 Rahimtoola S H Ehsani A Sinno M Z Loeb H S Rosen K M & Gunnar R M Left atrial transport function in myocardial infarction Importance of its booster pump function Am J Med 59 686 1975

14 Redwood D R Henry W L & Epstein S E Evaluation of the ability of echocardiography to measure acute alterations in left ventricular volume Circulation 50 901 1974

15 Sapoznikov D Weinman J & Eliakim M Left ventricular preejection period and pulse wave velocity during complete heart block and artificial pacing in man Eur J Cardiol 1 4 447 1974

16 Stefandouros M A & Canedo M J Reproducibility of echocardiographic estimates of the left ventricular dimensions Br Heart J 39 390 1977

17 Teichholz L E Kreulen T Herman M V & Gorlin R Problems in echocardiographic volume determinations Echocardiographic–angiographic correlations in the presence and absence of asynergy Am J Cardiol 37 7 1976

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Analgesic Treatment with Levomepromazine in Acute Myocardial Infarction

A Randomized Clinical Trial

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ABSTRACT The efficacy of a non narcotic analgesic is evaluated in a double blind randomized series of patients with acute myocardial infarction (AMI) Levomepromazine or pethidine were given in 328 consecutive cases to 316 patients within 24 hours after the onset of symptoms Levomepromazine 12 5 mg, appeared as effective as pethidine, 50 mg, in the alleviation of pain, though the initial dose had to be higher Nausea and vomiting were half as frequent in the levomepromazine group as in the pethidine group ($p<0\ 001$) The incidences of arrhythmias, lung oedema, hypotension and thromboembolic com plications did not differ between the groups The mortality rate in the first 4 weeks was 22% in the levomepromazine group and 37% in the pethidine group ($p<0\ 005$), and after one year 39 and 50% ($p<0\ 05$), respectively It is concluded that levomepromazine is better tolerated than pethidine in AMI This suggests that the present management of pain in AMI should be reconsidered

Key words analgesics myocardial infarction

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While other cardiac therapy has recently improved the management of pain in acute myocardial infarc tion (AMI) has remained unchanged The general consensus is that narcotic analgesics are the drugs of choice (1 5) In series of patients with AMI vomiting is frequently encountered (2) and respira tion is often compromised Analgesics without nauseating or respiration depressing effects may therefore be preferable to narcotics

Levomepromazine (methotrimeprazine Nozi nan® Veractil®) is a phenothiazine that has been used as a neuroleptic for more than 20 years with few side effects (9) Unlike other phenothiazines it is a potent analgesic (10 11) Like other pheno-thiazines it has a pronounced antiemetic effect (7) without untoward effects on the respiratory centre or the bronchi (13) It has seldom been used in MAI (11) and—after promising preliminary results —we planned a double blind randomized clinical comparison of levomepromazine with our tradi tional treatment with pethidine

PATIENTS

During 16 months 628 consecutive cases (603 patients) with a preliminary diagnosis of AMI causing symptoms for 24 hours or less were admitted to this series This report concerns 328 cases (316 patients) with definite myocar dial infarction (15) treated according to the plan

Exclusion a priori is due to known adverse reac tions to narcotics or phenothiazines treatment with levomepromazine before admission other acute disease and AMI in the last 4 weeks Three patients from either group were excluded because they died in 30 min after admission before treatment was expected to have any effect Five patients from either group were by error not treated according to the protocol No patient was ex cluded because of lack of analgesia

The averge age was 67 years in the levomepromazine group and 68 years in the pethidine group As to sex previous cardiac and hypertensive disease and clinical condition on admission there were only slight differences between the two groups (Table I) Fig I shows the dura tion of symptoms in the two groups before admission 50% of the patients in both groups were admitted within 3 hours

TREATMENT AND SCEDULE

Analgesics were prepared in standard doses of pethidine chloride 50 mg in vials and 100 mg in tablets and of levomepromazine chloride 12 5 mg in vials and le vomepromazine maleate 25 mg in tablets Tablets and vials of identical appearance were dispensed in numbered boxes from the pharmacy of Bispebjerg Hospital The sealed code was kept in the pharmacy and was not broken during the study

On admission the patient was allocated the first 1 able box When more than one box was n

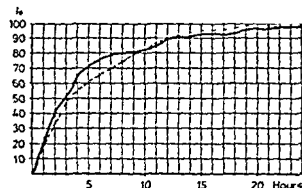


Fig 1 Duration of symptoms before entry — = Levomepromazine group — = pethidine group

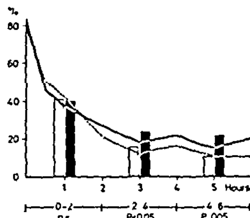


Fig 2 Pain disappearance curves and additional injections during the first 6 hours The curves show % of patients with pain the columns % of patients who were given additional injections □ = Levomepromazine group — ■ = pethidine group (n s $p > 0.05$ χ^2 test)

same content from the pool In accordance with the plan the statistician controlled the results after the first 100 cases with definite AMI More pethidine had been used than expected from the pilot series resulting in fewer cases in this group Consequently the boxes were prepared with more vials and an equal number of cases entered the groups during the last half of the investigation The patient received one injection immediately upon admission Further injections were given when needed for pain anxiety or lung oedema. One tablet was administered at 6 a.m 2 p m and 8 p m during the first 3 days This schedule was used to ensure that all patients received a sufficiently large total dose for evaluation of the systemic effect of the two drugs In the event of persistent vomiting or somnolence the oral dose was reduced to half in 8% of cases in the levomepromazine group and in 1% in the pethidine group A maximum dose of 9 tablets per patient was planned the mean consumption in the two groups was 8 4 and 8 3 tablets respectively

Nausea and vomiting were treated with diphenhydramine suppositories while other antiemetics analgesics tranquilizers and hypnotics were withheld Previous diuretic treatment was continued and additional therapy of heart failure was restricted to a high ceiling diuretic bumetanide (Burinex®)

All other treatment was given according to established routine Uncomplicated cases were mobilized from the first or second day and discharged after 2 weeks

Table I Distribution according to age sex history and clinical findings

	Levomepromazine group		Pethidine group	
	No	%	No	%
Total	185		143	
Age >65 y	112	61	94	66
Women	78	42	47	33
Previous hypertension	32	17	21	15
Previous heart insufficiency	73	39	63	44
Pain on admission	147	79	114	80
Lung oedema on admission	32	17	26	18
Cardiac arrest before entry	6	3	6	4
Treated before admission	65	35	45	31

$p > 0.05$ (χ^2 test).

OBSERVATION

ECG was monitored continuously during the trial Other intermittent observations were recorded at least every 30 min during the first hours and later at 6 a m 2 p m and 8 p m Patients asleep were woken up A record was kept about vomiting pain sleep nausea and moist skin A cuff BP was measured

The patients were seen in the Out Patient Clinic after 4 weeks

RESULTS

Pain was encountered during the course of the AMI in 90% of cases The incidence of pain was reduced to the same level in both groups and an identical number of doses of test drugs were given during the first two hours (Fig 2) suggesting an equipotent analgesic peak effect of 12 5 mg levomepromazine and 50 mg pethidine From the 2nd to the 6th hour pain was encountered more often and more injections were given in the pethidine than in the levomepromazine group This tendency was noted

Table II *Heart failure and arrhythmias during treatment*

	Levomepromazine group		Pethidine group		
	No	%	No	%	*p*
Total	185		143		
Systolic BP <80 mmHg	16	9	9	6	n s
Lung oedema	10	5	14	10	n s
Tachyarrhythmia	93	50	79	55	n s
Bradyarrhythmia	37	20	30	21	n s
Cardiac arrest	15	8	12	8	n s
Treated with digoxin	36	19	32	22	n s
Treated with lignocaine	43	23	36	25	n s
Treated with bumetanide	61	33	66	46	<0 05

n s =p>0 05 (χ^2 test)

throughout the period of treatment Recurrences of pain in the first 72 hours were observed in 50% of the levomepromazine treated and in 62% of the pethidine treated patients ($p<0\ 05$) Hence a mean of 4 injections of pethidine were required against a mean of 3 injections of levomepromazine

At standard observation time 49% of patients in the levomepromazine group were found asleep versus 23% in the pethidine group ($p<0\ 001$ χ^2 test) The incidences of nausea and vomiting were significantly higher ($p<0\ 001$) in pethidine treated than levomepromazine treated patients 56 versus 24% and 40 versus 17% respectively In keeping with this diphenhydramine was given to 41% versus 18% of the patients

The incidence of severe hypotension and lung oedema did not differ between the groups Neither did the number of patients treated with digoxin or antiarrhythmics The incidence of tachyarrhythmias and bradyarrhythmias showed no difference (Table II) Systolic BP (mean ± S D) on admission was for the levomepromazine group 150±38 mmHg and for the pethidine group 151±32 mmHg One hour later it was 131±29 and 137±30 mmHg respectively This fall in BP was not significant ($p>0\ 05$ Student s *t* test)

The incidence of hypotension did not differ between the groups during the first hour when medication was at its peak (Fig 3)

The incidence of dizziness mental disturbance and urinary retention was equal and totalled less than 8% in both groups The incidence of embolisms was 1% in both groups Dryness of the mouth which is particularly prominent in levomepromazine therapy appeared in this group in 59% of cases but also in 27% of the patients treated with pethidine ($p<0\ 001$) There was no tenderness at the injection site

The authors tried to estimate which analgesic had been administered to all patients who survived long enough to allow this observation Based on dryness of mouth nausea vomiting and incidence of sleep their guess was correct in 51% of the cases

The patients entered this study with a preliminary diagnosis of AMI to promote early treatment The

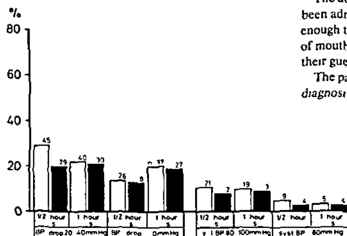


Fig 3 Drop in systolic BP and observation of low values within the first hour after the initial injection □=Levomepromazine group ■=pethidine group (n s $p>0\ 05$ χ^2 test)

diagnosis was settled after 1–3 days and may have been influenced by two different therapies However the four week mortality in all 603 patients admitted including all diagnoses and drop-outs was significantly lower ($p<0.05$) on levomepromazine (18% in 320 patients) than on pethidine treatment (25% in 283 patients) In patients without definite AMI the short term mortality was 11% on levomepromazine and 9% on pethidine i e without significant difference In patients with AMI the four week mortality 22% was significantly lower ($p<0.005$) in the levomepromazine group than in the pethidine group 37% More cases entered the levomepromazine group in the first half of the investigation This lopsidedness in selection did not influence the mortality in the two groups very much being 19 and 36% respectively, in the first 100 patients At one year follow up the difference was still present the mortality rate being 39 and 50% respectively ($p<0.05$) Also in the high risk groups i e above 65 years of age patients with previous heart failure lung oedema arterial hypertension and hypotension the mortality was lower in the levomepromazine group The mortality did not vary with duration of initial pain or with the number of recurrences of pain in either group

DISCUSSION

This study with clinical randomization confirms that levomepromazine is effective in the alleviation of pain in AMI The analgesic peak effect of 12 5 mg levomepromazine is comparable to that of 50 mg pethidine and the duration of the analgesia is longer The initial doses of the tested drugs are relatively small because some patients were treated before admission The pain disappearance curve (Fig 2) reveals that the initial dose of both drugs can be doubled with relative impunity

Our results indicate that levomepromazine is preferable to pethidine in AMI The antiemetic effect is particularly impressive it gives a better sedation without interfering with early mobilization and no serious adverse effects were encountered Furthermore it has no addictive properties (6)

The reason for the better survival on treatment with levomepromazine is not obvious The differences in age sex and case history are too small to account for the difference in mortality It may be related to an adverse effect of pethidine on the heart as described by others (12 14) or to a beneficial vasodilating effect of levomepromazine (7) or other vasodilating drugs (3, 4 8)

If the last presumption is confirmed the benefit of vasodilating therapy can be obtained with simple measures and without the need for invasive hemodynamic control

REFERENCES

1 Alderman, E L Analgesics in the acute phase of myocardial infarction JAMA 229 1646 1974
2 Bjerkelund C Nitter Hauge S & Jakobsen E Perphenazine (Trilafon®) in the prophylaxis of nausea and vomiting following acute myocardial infarct Act Med Scand 177 7-9 1965
3 Borer J S Redwood D R Levitt B Cagin N Bianchi C Vallin H & Epstein S E Reduction in myocardial ischemia with nitroglycerin or nitroglycerin plus phenylephrin administered during acute myocardial infarction N Engl J Med 293 1008 1975
4 Editorial Hypotensive treatment for acute myocardial infarction Br Med J 1 353 1975
5 Editorial Pentazocine in myocardial infarction Lancet 2 868 1976
6 Fraser H F & Rosenberg D E Observations on the human pharmacology and addictiveness of methotrimeprazine Clin Pharmacol Ther 4 596 1963
7 Goodman L S & Gilman A The pharmacological basis of therapeutics p 157 Macmillan New York 1975
8 Gould L Reddy C V R Phentolamine Am Heart J 92 397 1976
9 Henne M Henne S Kop S & Tonnel M Observations on levomepromazine treatment on 50 hospitalized patients over 5 years Proc 3rd World Congr Psych p 976 University of Toronto Press, Montreal 1961
10 Maxwell D R Palmer H T & Ryall R W A comparison of the analgesic and some other central properties of methotrimeprazine and morphine Arch Int Pharmacodyn Ther 132 60 1961
11 Montilla E Frederik W S & Cass L J Analgesic effect of methotrimeprazine and morphine Arch Intern Med 111 725 1963
12 Mostert J W Evers J L Hobika G H Moore R H & Murphy G P Circulatory effects of analgesic and neuroleptic drugs in patients with chronic renal failure undergoing maintenance dialysis Br J Anesth 42 501 1970
13 Pearson J W & De Kornfeld T J Effect of methotrimeprazine on respiration Anesthesiology 24 38 1963
14 Strauer B E Herzwirkung des Pethidins Intensivmedizin 12 312 1975
15 WHO Ischemic heart disease registers Report of the fifth working group Copenhagen 1971

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Impact of a Mobile Coronary Care Unit on the Sudden Coronary Mortality in a Community

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ABSTRACT A 6-month feasibility study on a mobile coronary care unit (MCCU) was implemented in an urban community provided with a WHO Acute Ischemic Heart Disease Register MCCU was able to reach in time less than 5% of all cases of unexpected cardiac arrest in the community For cases of myocardial infarction and cardiac arrest transported by MCCU, a pair matched control series was obtained from a period of 2–8 months before the beginning of the MCCU activity No difference was found in the first 28 days mortality rate between MCCU and control groups The operation of MCCU did not induce any reduction of the patient delay time in the community

Key words acute myocardial infarction cardiac resuscitation coronary ambulance mobile coronary care unit sudden coronary death

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The case fatality rate in acute ischemic heart attack is close to 40% in many countries with a high incidence of coronary heart disease (21 22) About two thirds of this high rate are attributed to deaths outside hospitals (10 15) The maximum possible reduction in community coronary deaths that can be achieved with the aid of hospital coronary care units (CCU) is therefore only 3–5% (14) Thus it is not surprising that the encouraging results in prehospital coronary care reported by Pantridge and Geddes (18) evoked a great deal of enthusiasm for mobile coronary care units (MCCU) On the other hand because one half or even more of prehospital deaths occur more or less instantaneously doubts have been expressed as to whether prehospital mortality can be reduced by emergency therapeutic means such as MCCU (6 9 19) In the last ten years numerous reports of experiences with MCCU have appeared in the literature with conclusions varying from optimism (2 3) to pessimism (4 12) No report includes data on the impact of MCCU on the total coronary mortality of the community served by the unit and the evaluation of the usefulness of MCCU is therefore difficult

The present paper reports the results of a feasibility study on MCCU implemented in Helsinki in 1971 Due to the existence of an Acute Ischemic Heart Disease Register (AIHDR) operating in this area it was possible to collect data necessary for evaluating the importance of MCCU in prevention of sudden coronary death

METHODS

The feasibility study of MCCU was carried out over a 6-month period beginning on March 1 1971 by the Finnish Heart Association in cooperation with the health authorities of the City of Helsinki and the Helsinki University Central Hospital The MCCU was manned with a driver a nurse and a medical student all volunteers and all well trained in cardiopulmonary resuscitation On each dispatch from the base which was situated in the vicinity of the University Central Hospital the MCCU picked up a physician from the hospital CCU which was alerted simultaneously with the MCCU base The MCCU was provided with a portable ECG monitor and DC defibrillator equipment for endotracheal intubation and ventilation and a complete set of parenteral drugs and infusion fluids for cardiovascular emergency care The MCCU was dispatched by call by an experienced nurse who was on duty at the city emergency service and who made the decisions independently Both the emergency service and the MCCU worked 24 hours a day Anybody could contact the emergency service but not the MCCU itself The

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Abbreviations CCU=coronary care unit MCCU=mobile CCU AIHDR=Acute Ischemic Heart Disease Register AIHD=acute ischemic heart disease AMI=acute myocardial infarction VF=ventricular fibrilla

Table I Validity of pair matching

	MCCU group		Control group	
	N	%	N	%
Size of infarction				
ECG				
Transmural	49	74	56	75
Non transmural	17	26	19	25
Not known	24		15	
Anterior	36	55	39	52
Inferior	29	45	36	48
Not known	25		12	
Enzymes				
Elevated	64	79	62	75
Equivocal	8	10	4	5
Normal	9	11	17	20
Not known	9		7	
Peel s index				
1	39	48	36	45
2	22	27	11	14
3	11	13	15	19
4	10	12	18	22
Not known	8		10	
Reliability of diagnosis				
Chest pain				
Typical	69	83	79	89
Atypical	6	7	8	9
No pain	8	10	2	2
Not known	7		1	
ECG				
Unequivocal	60	75	48	57
Equivocal	15	19	24	28
No infarction other changes	4	5	11	13
No changes	1	1	2	2

emergency telephone number was generally known On dispatch the MCCU had a radio telephone connection to the base to the hospital CCU to the patient s home and to the dispatcher on duty and was able to send an ECG to the CCU by radiotelephone connection if necessary by utilizing a device based on an acoustic carrier wave system During rush hours a police squad was also dispatched to clear the streets All pertinent times events therapeutic measures and medical observations were recorded on a special form

In the MCCU the use of the same methods of coronary care as in the hospital CCU was natural because both units had the same physicians During the study period no essential changes occurred in these methods in the hospital CCU in the MCCU or among the general practitioners in the city

An AIHDR had been operating in the same area since Sept 1969 The AIHDR collected medical and other pertinent information on all cases of acute myocardial infarction (AMI) and sudden coronary death occurring in people younger than 65 in the City of Helsinki The design and function of the AIHDR are presented in detail elsewhere (19 20 22)

PATIENT SERIES

The study group consists of 233 cases of acute ischemic heart disease (AIHD) i e AMI and cardiac arrest who fulfilled the criteria of the WHO for registry by the AIHDR as a case of AIHD and were managed by the MCCU during the study period March 1–Aug 31 1971 The distribution by sex was unequal as compared with all cases of myocardial infarction or cardiac arrest in the community because the male/female ratio of the patients under 65 years of age was 1 78 whereas it was 3 03 in the AIHDR during the same year The age distribution corresponded roughly to that of all cases in the area but the older age groups were slightly overrepresented in the study group

For an analysis of the net effect on mortality a subgroup of 90 patients was formed from the study group by excluding patients aged 65 or more and also patients who in all probability were dead when the MCCU base received the call A control group of the same size was formed from AIHDR files covering the period July 1–Dec 31 1970 i e a period when the MCCU was not yet in operation The control group was formed from consecutive entries of the AIHDR by pair matching For every test subject was chosen a pair fulfilling the following conditions same sex equal age equal patient delay equal means of contacting emergency service alive when ambulance or MCCU was dispatched The validity of the matching was tested by comparing some pertinent clinical characteristics of both groups and the results are shown in Table I The study group and the control group were very similar the only difference being slightly more frequent unequivocal ECG changes in the study group

RESULTS

Time intervals and delays

The medians of different time intervals and delays in operating the MCCU vs an ambulance are given in Table II The data for ordinary ambulance were obtained from a one year period from Sept 1970 to Aug 31 1971 The main characteristics of the MCCU activity were longer median total delay i e the time interval from the onset of symptoms to admission to hospital longer duration of transportation and shorter delay in starting specific treatment

Table II Median delay time intervals (min)

	MCCU	Controls
Total delay	92	85
Patient delay	50	65
MCCU (ambulance) delay	7	6
Delay of therapy	57	71
MCCU (ambulance) occupied	42	22
Duration of transportation	35	16

Table III *Distribution of transportations according to heart disease category*

	N	%
Total no of transportations	791	
Heart disease	588	74
Coronary heart disease	452	57
Myocardial infarction	233	29
Cardiac arrest	60	8
Cardiac arrest alive	25	3

Transportation

The MCCU was dispatched 812 times during the study period of 184 days i e 4 4 times per day Due to insufficient data 21 dispatches were discarded and thus data on 791 dispatches were subjected to more detailed analysis The distribution of transportations according to different heart disease categories is given in Table III which gives an idea of how well the emergency service was able to classify the emergency calls into those dealing with cardiacs and those dealing with non cardiacs According to the AIHDR files during the test period only 7 cases of AIHD were considered non cardiac by the emergency service In 11 additional cases MCCU was not dispatched because the unit was occupied by another case Altogether 233 cases of AMI were managed by this system during the study period This is slightly more than one out of six cases of AMI or cardiac arrest occurring daily in Helsinki

Table IV *Clinical condition and survival of patients*

	N	%
Clinical condition on arrival of MCCU		
Dead	38	15 2
Alive		
Cardiac arrest	25	10 8
Asystole	2	0 8
VF	23	9 9
VPBs	33	14 2
Bradycardia	11	4 7
Tachyarrhythmias	16	6 9
Hypotension	38	16 3
Heart failure	35	15 0
Survival rate of those 25 alive on arrival of MCCU		
On admission to hospital	21	84
24 hours	14	56
28 days	8	32
3 months	8	32
12 months	8	32

Table V *Complications during a 4 week period and mortality during 24 hours and 28 days*

	MCCU group		Control group	
	N	%	N	%
Complications				
Heart failure	59	71	46	51
Hypotension	19	23	4	4
Cardiac arrest	18	22	6	7
Reinfarction	0	0	1	1
Angina pectoris	3	4	18	20
Thromboembolism	1	1	0	0
Other	7	12	10	11
Mortality				
24 h	9	10	07	08
28 d	24	27	20	22

Resuscitation

The patients clinical condition on arrival of the MCCU is given in Table IV In all cases of cardiac arrest this condition already existed at the time of the first medical examination immediately after arrival Cardiopulmonary resuscitation resulted in restoration of the cardiac pump function in all cases of ventricular fibrillation (VF) Thus there were 23 successful resuscitations The survival figures of these cases up to one year s follow up are shown in Table IV Resuscitation was unsuccessful in the two cases of asystole

Impact on clinical course and mortality

The only difference in complications between the groups was more frequent hypotension in the MCCU group (Table V) In the MCCU group however the figures include also transient episodes of hypotension registered during transportation contrary to the controls whose blood pressures were recorded first in the hospital The average duration of hospital care was slightly longer in the MCCU group than in the control group A comparison of the 24 hour and 4-week mortality figures is also given in Table V

Impact on public behavior in cardiac emergencies

The MCCU activity and the very w
publicity it received in the media duri

period were expected to influence the behavior of the population in cardiac emergencies This was studied simply by monthly monitoring of the patient delay time in all cases of AIHD occurring in the study area The patient delay time was monitored monthly also before the study period and was found to be very stable (Jan 60 Feb 62 min) It remained the same over the start of MCCU activity on March 1 (March 62 April 65 May 65 min) and even increased at the beginning of the summer vacation months (June 105 min)

DISCUSSION

The total delay from the onset of symptoms of AIHD to hospital admission in Helsinki is one of the shortest in Europe i e about two hours and the patient delay constitutes more than one half of the total delay of hospitalization (22) Not much impact on the different delays and time intervals was thus expected from MCCU activity However because 15% of all deaths in Helsinki during the first 28 days after the onset of the attack occurred during transportation prior to start of MCCU activity (20) a reduction in the delay in starting specific treatment was one of the desired effects In fact some reduc in delay was achieved On the other hand in te of the modest size of Helsinki (greatest diame 17 and 18 km area 177 km²) the median time interval from emergency call to arrival of the MCCU varied considerably between different city districts i e in many districts it was impossible to reach cases of cardiac arrest in time Considering the whole city area 50% of all cases were reached in 7 min on average 75% in 11 min and 95% in 16 min Thus a rather limited proportion of cases of cardiac arrest were within reach of the MCCU in proper time The present series however demonstrated that resuscitation was successful in the majority of patients who were alive at the time medical aid was summoned but had VF when the MCCU arrived Hence the success of the MCCU seems to depend particularly on how early medical aid is summoned

The ability of the city emergency service to reach the cardiac emergencies was as a whole very limited The obvious principal reason for this is not related to the organization itself It is evident that in only one tenth of cases of cardiac arrest are any measures taken to summon medical aid (19 23) It can be calculated on the basis of this fact and the known incidence of primary VF outside hospitals in Helsinki (19) that not more than 30 cases of cardiac arrest should have been found by the MCCU during the study period In fact resuscitation was started in 63 patients but 38 of them proved to be clinically dead when the MCCU arrived The AIHDR files revealed that the majority (86%) of those cases of cardiac arrest in which medical aid was summoned were among these 63 but only half of them were clinically alive when the MCCU arrived In the light of these figures the MCCU was able to reach about half of the cases with cardiac arrest in which medical aid was summoned in time which is less than 5% of all cases of unexpected cardiac arrest However the overall sensitivity of the emergency service was better than one would expect on the basis of these figures because 45% of all cases were transported directly to a hospital by transports other than ambulance and without alerting the city emergency service

All patients with VF who were clinically alive when the MCCU arrived were successfully resuscitated One third of the resuscitated patients survived four weeks after the onset of symptoms and the same patients were still alive at the one year follow up The primary survival of 84% at the time of hospital admission is close to that reported by Pantridge and Adgey (17) The hospital mortality however was rather high and resulted in a survival rate at discharge close to that of MCCU patients reported by Liberthson et al (12) and slightly higher than that of 19 1% reported for patients resuscitated from cardiac arrest during hospital care (11) On the other hand no more deaths occurred after discharge up to one year s follow up In the light of these figures two different subgroups seem to exist among the resuscitated patients one with a poor and one with a definitely better prognosis Due to the small size of the groups no conclusions can be drawn on their characteristics The ratio of the number of rescues to months of MCCU activity was 1 3 which is three times that in Brighton and 5 times that in Nottingham (7) Thus in terms of rescues the efficiency of the MCCU seemed to be rather good

There are no reports in the literature regarding the net effect of the MCCU on the community coronary mortality This is not surprising because there are many problems in implementing such a study e g random allocation of patients into management by an MCCU or an ordinary ambulance

etc In the present study comparisons were made between MCCU patients who were under 65 years of age and alive at the time medical aid was summoned and their matched pairs from a similar period 2–8 months earlier Both groups included nearly the same number of patients with primary infarction (66 and 65% respectively) and did not differ clinically in other respects either Continuous monitoring by AIHDR of different delays the fatality of the ischemic attack hospital mortality etc in the whole community over the years 1970–71 revealed no essential or systematic changes in these circumstances Consequently there are good reasons to consider these two groups comparable The number of deaths during the first four weeks after the onset of symptoms was not dissimilar in the two groups which indicates that the MCCU had no measurable effect on the final mortality figures Further the number of deaths in the MCCU group also included 17 patients who had cardiac arrest on the arrival of the MCCU and were resuscitated but either did not recover or died during the first 28 days The remaining 8 survivors of the resuscitated group could be considered potentially dead in this context because without resuscitation their cardiac arrest would have been irreversible in all probability Then the total mortality in the MCCU group would be 32 out of 90 i e 35% This is a rather high mortality figure and slightly higher for instance than the mortality of all patients with myocardial infarction who were transported in Helsinki in 1970 by ordinary ambulance Thus the net effect of the MCCU on the early mortality in AMI and unexpected cardiac arrest seems to be negligible in addition there was some overmortality in the MCCU group concealed by the therapeutic activity of the MCCU

It is not easy to find the reason for the small but clear overmortality in the MCCU group Firstly the series is so small that the phenomenon may be due to mere chance Further in spite of careful matching the groups may still have been different with regard to their clinical severity A slightly higher incidence of some complications in the MCCU group suggests that there might be a real but small difference between the groups However these small differences hardly fully explain the differences in mortality The condition of surviving until medical aid was summoned is crucial in the allocation of patients into the subgroups of mortality comparison However a possible bias in this procedure should randomly affect both groups because of uniform criteria in interpreting circumstances and events found in AIHDR interviews Resuscitation may sometimes be started unnecessarily but on the basis of clinical documents it had not been done in the MCCU series

One explanation for the high mortality in the MCCU group would be apprehension due to the many exceptional measures inherent in the approach of the rescue squad It is well known that in patients with myocardial infarction fear and apprehension increase the incidence of malignant ventricular arrhythmias The possibility of precipitating VF by the activities of the MCCU has been disputed because more than 80% of VF managed by the MCCU occur before arrival of the unit (16) In fact in all cases of VF in the present series the arrhythmia was already present when the MCCU arrived but this does not necessarily mean that there is no association between the approach of the rescue squad and the excess mortality For instance it has been shown that during hospital ward rounds sudden death in AMI patients is more prevalent than could be expected by chance (8)

The most important component of total hospitalization delay in Helsinki is the patient decision time Its length seems to be very similar to that in many other centers about one hour irrespective of the duration of the total delay (15 17 22) Its reduction would be most desirable in order to shorten the hospitalization delay and this was expected to happen as a consequence of the intensive discussion of the topic in the media during the study period However the findings were not very encouraging After start of the MCCU activity there was not even a slight shortening of the patient delay time Apparently a reduction of the patient s decision time is not within the capability of ordinary public information Even prolonged operation of the MCCU has not been found to affect much this stable time interval (17)

The MCCU clearly has an important role as one of the measures for preventing sudden death However its net impact on the total community coronary mortality seems to be small in an area where the total hospitalization delay is small Its importance may be greater in communities where great distances traffic etc cause a marked delay in hospitalization because under these circ[illegible]st nces the MCCU shortens the initiati[illegible] therapeutic measures Great care

imperative in MCCU activity otherwise the beneficial results may be cancelled out by adverse effects

As stressed by many authors (1 5 6 10 13) the greatest emphasis in measures for reducing the incidence of sudden death should be put on identifying the risk subjects for sudden death providing them with first aid drugs and/or with long term preventive drug therapy and educating people in some appropriate way in cardiopulmonary resuscitation

PEFERENCES

1 Bondurant S Problems of the pre-hospital phase of acute myocardial infarction Am J Cardiol 24 612 1969
2 Cobb L A & Alvarez, H Services for cardiovascular emergencies WHO Technical Report Series no 562 Geneva 1975
3 Crampton R S Aldrich R R & Gascho J A Treatment of acute myocardial infarction Lancet 1 1106 1974
4 Dewar H A McCollum J P K & Floyd M A year's experience with a mobile coronary resuscitation unit. Br Med J 4 226 1969
5 Erhardt L R. Sjögren A & Sawe U Hur skall vi få hjartinfarktpatienten snabbare till sjukhus? Lakartidningen 72 4273 1969
6 Gulum R. F Feinleib M Margolis J R., Fabritz, R. R & Brasch R. C Delay in the prehospital phase of acute myocardial infarction Arch Intern Med 136 6·3 1976

J R Dowling, M & Nicholas C Comparison of results from a cardiac ambulance manned by medical or non-medical personnel Lancet 1 526 1977
8 Jarvinen F A J Can ward rounds be a danger to patients with myocardial infarction? Br Med J 1 318 1955
9 Julian D G Coronary care and the community Ann Intern Med 69 607 1968
10 Kuller L Sudden death in arteriosclerotic heart disease The case for preventive medicine Am J Cardiol 24 617 1969
11 Lemire J G & Johnson A L Is cardiac resuscitation worthwhile? A decade of experience N Engl J Med 286 970 1972
12 Liberthson R R Nagel E L Hirschman J C & Nussenfeld S R Prehospital ventricular defibrillation Prognosis and follow-up course JAMA 291 317 1974
13 Lown B & Ruberman W The concept of precoronary care Mod Concepts Cardiovasc Dis 39 97 1970
14 Lown B Klein M D & Hershberg, P I Coronary and precoronary care Am J Med 46 705 1969
15 McNeilly R. H & Pemberton J Duration of last attack in 998 fatal cases of coronary artery disease and its relation to possible cardiac resuscitation Br Med J 3 139 1966
16 Pantridge J F Prehospital coronary care (Editorial) Br Heart J 36 233 1974
17 Pantridge J F & Adgey A A J The pre-hospital phase of acute myocardial infarction In Textbook of coronary care (ed L E Meltzer & A J Dunning) pp 95–106 Excerpta Medica Amsterdam 1972
18 Pantridge J F & Geddes J S A mobile intensive care unit in the management of myocardial infarction Lancet 1 271 1967
19 Romo M Factors related to sudden death in acute ischaemic heart disease A community study in Helsinki Acta Med Scand (Suppl) 547 1972
20 Siltanen P The ischaemic heart disease register as a frame for preventive measures Adv Cardiol 8 214 1973
21 Weinblatt E Shapiro S Franck C W & Sager R. V Prognosis of men after first myocardial infarction. Mortality and first recurrence in relation to selected parameters Am J Public Health 58 1329 1968
22 WHO Regional Office for Europe Myocardial infarction community registers Public Health in Europe no 5 Copenhagen 1976
23 Wikland B Medically unattended fatal cases of ischaemic heart disease in a defined population Acta Med Scand (Suppl) 524 1971

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Differences in Metabolic Responses to β-Adrenergic Stimulation after Propranolol or Metoprolol Administration

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ABSTRACT Isoprenaline, or the β_2-agonist terbutaline, was infused in healthy male volunteers and the plasma levels of insulin, glucose and free fatty acids (FFA) were determined Saline, propranolol or *the selective β_1-receptor antagonist metoprolol*, was administered i v prior to the infusion of the β-stimulants The two β-receptor blockers inhibited isoprenaline-induced increase in chronotropy to about the same extent, while the effects on systolic and diastolic blood pressure were in accordance with a selective β_1-blocking effect of metoprolol and a non-selective β-blocking action of propranolol Quantitative differences were found between metoprolol and propranolol on the metabolic parameters The effects can best be described in terms of β_1- or β_2-receptors where effects on plasma FFA and glycerol levels seem to be mainly β_1-mediated An apparent β_2-mediated effect was found for insulin release and hepatic glucose output

Key words β-adrenergic receptors insulin FFA glycerol metoprolol propranolol

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The *metabolic effects of β receptor-stimulating* agents and β blocking agents concerning insulin release glucose homeostasis and lipolysis have been studied in recent years both in vitro and in vivo (3 8 10 17 26)

Although the main insulin regulating mechanism is the blood glucose levels it seems well established that adrenergic β-receptors may in some way be involved in insulin release (17) Cerasi et al (8) have suggested that glucose stimulated insulin release is associated with the β-receptors while studies by Robertson and Porte (26) conflict with this concept and distinguish between a glucose receptor and a β receptor

Loubatieres et al (24) demonstrated a stimulating effect of isoproterenol on insulin secretion in the dog This effect was inhibited by propranolol but *not by practolol a specific β_1 adrenoceptor blocking* compound Furthermore they found that salbutamol a selective β_2 receptor stimulant provoked the release of insulin and this effect was blocked by propranolol Their findings have been confirmed by Kaneto et al (22) However studies in man characterizing the type of β receptor present in the pancreas do not appear to have been carried out

The effect of β adrenergic blockade on the release of different substrates is also unclear (10) although it may have important consequences for carbohydrate and lipid metabolism The relative effects of β-blocking drugs with differing specificity should therefore be evaluated

The present investigation was designed to study the effects of propranolol and the selective β_1-receptor blocker metoprolol given prior to the β stimulating agent isoprenaline or the selective β_2 *stimulator terbutaline on the plasma levels of* insulin glucose free fatty acids (FFA) and glycerol

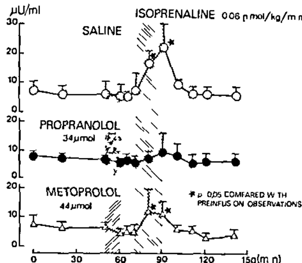
SUBJECTS AND METHODS

Eight healthy men aged 27–42 years (mean 32) and weighing 67–97 kg (mean 82) with normal oral glucose tolerance test and without heredity for diabetes were studied after an overnight fast They had normal blood lipids except for one subject who was overweight and who had blood lipids classified as type IIB hyperlipoproteinemia

The volunteers were in the recumbent position with catheters inserted in each cubital vein one for blood sampling and one for the infusions The investigation period was 140 min. Pulse and blood pressure (BP) were followed

Blood samples were drawn at the indicated times and *analyzed for insulin by a radioimmunoassay technique* (Phadebas* Pharmacia Uppsala Sweden) and for glu

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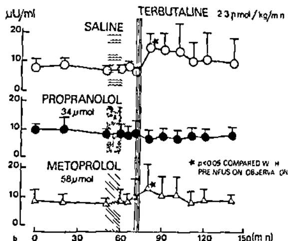


Fig 1 Plasma insulin levels in response to isoprenaline infusions in 5 healthy males (*a*) and to terbutaline infusions in 8 healthy males (*b*) following administration of the indicated agents (mean ± S D *$p<0\ 05$)

cose (4) FFA (18) and glycerol (13) Three blood samples taken 50 min before the infusions were used as controls of the non stimulated state

The isoprenaline study comprises five subjects During the period between 50 and 60 min 34 μmol (10 mg) of propranolol or 44 μmol (15 mg) of metoprolol were infused Saline served as control Between 70 and 90 min 06 μmol/kg b wt /min (0 015 μg/kg b wt /min) of isoaline was infused

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In both studies the effect of the different agents was investigated in the same subject At least two days elapsed between each investigation The statistical evaluation of the results was made according to Student s t test and a value of probability of $p<0\ 05$ was considered statistically significant

RESULTS

Isoprenaline infusion

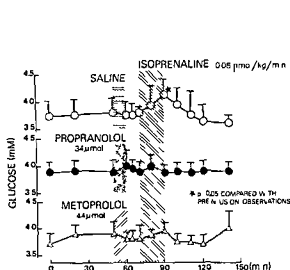
Hemodynamic changes During isoprenaline infusion the pulse rate increased about 30% This effect was completely blocked by both propranolol and metoprolol

When only saline was given systolic BP rose and diastolic BP fell during the isoprenaline infusion The isoprenaline induced increase in systolic BP was blocked to about the same extent by the two β receptor blockers However the lowering effect on the diastolic BP was completely inhibited by propranolol while this effect could still be observed with metoprolol Thus these findings show that the β blocking doses used produced similar blockade of heart rate and thus of the β_1 receptors However the relative β_1 selectivity of metoprolol was still maintained

Changes in blood levels of hormones and substrate Isoprenaline caused a threefold increase in the insulin levels (Fig 1*a*) This effect was entirely blocked by propranolol (Fig 1*a*) but with metoprolol a twofold increase could still be seen (Fig 1*a*) Isoprenaline slightly raised the glucose levels (Fig 2*a*) This effect was entirely blocked by propranolol (Fig 2*a*) but some slight increase was found with metoprolol (Fig 2*a*) although it did not reach statistical significance The isoprenaline induced increase (about 100%) in FFA levels (Fig 3*a*) was completely inhibited by propranolol while there was still a significant increase (about 40%) during metoprolol blockade (Fig 3*a*) Essentially the same results were obtained with the blood glycerol levels

Terbutaline infusion

Hemodynamic changes During infusion of the β_2-receptor agonist terbutaline the pulse rate increased about 20% and remained 10% over the basal state even after the infusion of terbutaline had ceased Propranolol decreased the pulse rate about 10% and effectively blocked the effect of



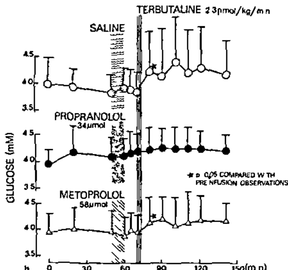


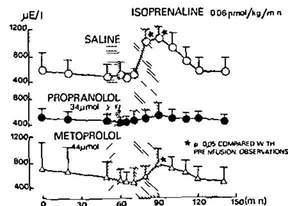
Fig 2 Plasma glucose levels in response to isoprenaline infusions in 5 healthy males (*a*) and to terbutaline infusions in 8 healthy males (*b*) following administration of the indicated agents (mean + S D *$p<0.05$)

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Terbutaline alone caused a slight increase in systolic BP and a drop in diastolic BP Propranolol alone did not affect the BP but completely blocked the terbutaline induced changes Metoprolol affected the terbutaline induced changes in BP less than propranolol although the decrease in diastolic BP did not reach statistical significance Again these hemodynamic effects suggest that at the dose used the relative β_1 selectivity of metoprolol was maintained

Changes in blood levels of hormones and substrates The dose of terbutaline used increased the insulin levels about two times and elevated levels were found throughout the period of observation (Fig 1*b*) Propranolol (Fig 1*b*) completely blocked this effect while metoprolol (Fig 1*b*) modified the terbutaline effect to about a 50% increase

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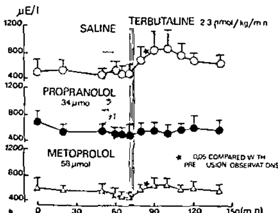
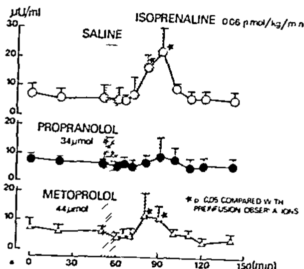


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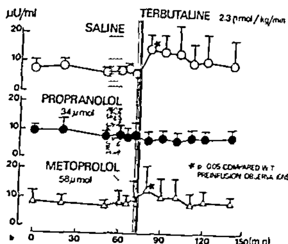


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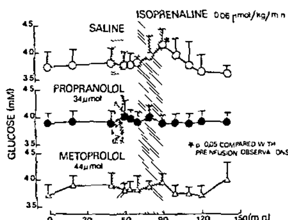
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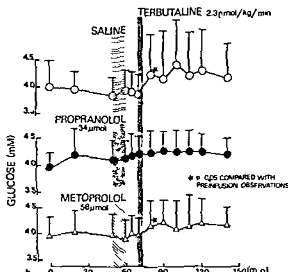


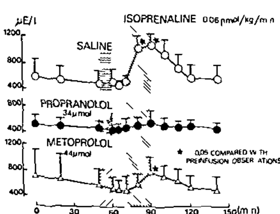
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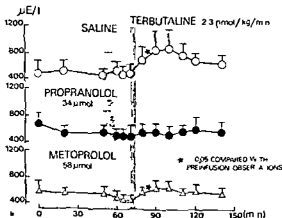


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blocked by propranolol (Fig 2*b*) while significantly increased glucose levels compared to the basal state were still found after metoprolol administration (Fig 2*b*)

Terbutaline increased the FFA levels (Fig 3*b*) This effect was again completely blocked by propranolol (Fig 3*b*) but not metoprolol (Fig 3*b*) Essentially the same results were obtained with the blood glycerol levels During infusion of propranolol alone (Fig 3*b*) the FFA levels were not significantly affected while metoprolol (Fig 3*b*) caused a significant decrease

DISCUSSION

In several countries β adrenergic blocking agents have gained widespread use in the treatment of cardiovascular disorders such as angina pectoris and/or hypertension However β receptors are not only localized in the heart or in the vessels but mediate several important metabolic effects The metabolic aspects of treatment with β-blocking agents have attracted considerably less interest from a research point of view than the cardiovascular effects (10) The present study was designed to investigate the type of β receptor (β_1 or β) mainly lved in adrenergic stimulated insulin release mobilization and glycogenolysis Knowledge the type of β receptor involved is of clinical importance since both non selective and cardioselective β_1 blocking agents are now available for patient treatment

A primary basis for characterizing the receptors as of β_1- or β_2-type is the construction of dose–response curves with different selective agonists and antagonists However the possible existence of both β_1 and β_2-receptors within the same tissue although in different proportions can make even this evaluation difficult (6) Consequently clinical studies such as the present investigation do not permit safe conclusions regarding the exact population of the β_1 or β_2 receptors involved in the metabolic processes studied However at therapeutic dose levels and and doses with equal β_1 blocking properties certain differences were found between the effects of metoprolol and propranolol on isoprenaline induced increases in chronotropy

The isoprenaline dose was chosen in accordance with previous studies (24) and gave a clear effect on pulse rate and BP Contrary to isoprenaline with its rapid turnover requiring constant infusion the turnover of terbutaline made it possible to give this drug as a single dose The terbutaline dose was chosen to give as specific β_2 stimulation as possible (2) Since the receptor specificity is dose dependent also this evaluation becomes difficult without the combination of a β_1 blocker such as metoprolol

The results on pulse rate and BP are in agreement with previous studies (20 21) demonstrating that propranolol acts indeed as a non selective and metoprolol as a β_1 selective blocker

The effects of β-adrenergic stimulation/inhibition on plasma glycerol and FFA levels which at least partly reflect the rate of adipose tissue lipolysis are well known and appear to be the result of mainly β_1-effects although a β effect also appears to be present (15) Also direct in vitro studies have shown that lipolysis is mainly a β_1-effect although β_2-stimulating agents can also elicit a clear lipolytic response (19) The presence of a β_1-effect was demonstrated by the observation that metoprolol largely blocked the isoprenaline induced increase in FFA and glycerol The β_2 influence was demonstrated in the experiments with both metoprolol and terbutaline where slightly elevated plasma FFA levels were still found

In addition the present results indicate that β_2-receptors can to some extent contribute to adrenergic mediated glucose output as also previously suggested (7) Thus in contrast to metoprolol it was found that propranolol blocked both isoprenaline and terbutaline induced increases in blood glucose levels However it seems unlikely that this finding is of sufficient quantitative importance to explain the observation that insulin induced hypoglycemia is prolonged if patients are simultaneously treated with propranolol (1 23) while a more normal recovery is obtained with a selective β_1 blocking agent (9 11) Several hormones are involved in the recovery of a hypoglycemia although it seems that catecholamine mediated effects are particularly important (14) It may well be then that the difference in recovery after hypoglycemia between β_1 selective and non selective β blocking drugs may be due to differences in the release and effect of important gluconeogenic hormones rather than to differences in β mediated glycogenolysis In any case it appears quite clear that a selective β_1 blocking agent may offer advantages over a non selective

drug for patients who may have hypoglycemic attacks such as those treated medically for diabetes mellitus or patients with a low glycogen reserve A recommendation not to use non selective β blocking drugs in such patients has also recently been given (12)

Adrenergic insulin release seems to be mediated by both β_1 and β receptors similar to the β adrenergic control of lipolysis However since the blood glucose levels were slightly increased by the β adrenergic agonists used in the present study it cannot be ruled out that the rise in insulin levels found was secondary to this effect It is well known that small increases in blood glucose stimulate insulin secretion in healthy subjects Without knowledge of the arteriovenous differences of insulin and glucose in the peripheral tissues and in pancreas it is impossible to draw definite conclusions about the direct β adrenergic control of insulin release However in three subjects in whom blood glucose and insulin were quantified immediately after the terbutaline infusion the insulin rise was seen before the glucose rise and the changes in blood FFA Thus it seems that β_2-receptors are present in human pancreas However long term treatment with non selective β blocking agents does not usually lead to a reduced glucose tolerance (16 27) On the other hand reports have appeared clearly showing that in some subjects glucose tolerance as well as insulin release are reduced by non selective β blocking agents (25 28) and this effect can be reversed by treating the same patients with a selective β_1 blocker (28) In fact in patients with an insuloma this antagonistic effect of a non selective β blocking agent on insulin release has been used therapeutically (5) These findings support the concept that β receptors are involved in that part of the insulin release which is associated with the β receptors However further studies are required in different patient groups in order to evaluate the possible clinical importance of the β-receptor associated insulin release

REFERENCES

1 Abramson E A Arky R A & Woeber K A Effects of propranolol on the hormonal and metabolic responses to insulin induced hypoglycaemia Lancet 2 1386 1966

2 Arner B Bertler Å Karlefors T & Westling H Circulatory effects of orciprenaline adrenaline and a new sympathomimetic β receptor stimulating agent terbutaline in normal human subjects Acta Med Scand (Suppl) 512 25 1970

3 Arnold A Differentiation of receptors activated by catecholamines III Il Farmaco Ed Sci 27 79 1972

4 Bergmeyer H V Bernt E Schmidt F & Stork H D-Glucose Bestimmung mit Hexokinase und Glucose-6-Phosphatase Dehydrogenase In Methoden der enzymatischen Analyse (ed H V Bergmeyer) p 1241 Verlag Chemie Weinheim 1974

5 Blum J Doron M Laron Z Atsmon A & Tiqva P Prevention of hypoglycemic attacks by propranolol in a patient suffering from insulinoma Diabetes 24 535 1975

6 Carlsson E Åblad B Brandstrom A & Carlsson B Differentiated blockade of the chronotropic effects of various adrenergic stimuli in the cat heart Life Sci 11 953 1972

7 Carlstrom S & Westling H Metabolic circulatory and respiratory effects of a new sympathomimetic β-receptor stimulating agent terbutaline compared with those of orciprenaline Acta Med Scand (Suppl) 512 33 1970

8 Cerasi E Luft R & Efendic S Effect of adrenergic blocking agents on insulin response to glucose infusion in man Acta Endocrinol (Kbh) 69 335 1972

9 Davidson N McD Corall R J M Shaw T R D & French E B Observations in man of hypoglycaemia during selective and non selective beta blockade Scot Med J 22 69 1977

10 Day J I The metabolic consequences of adrenergic blockade A review Metabolism 24 987 1975

11 Deacon S P & Barnett D Comparison of atenolol and propranolol during insulin-induced hypoglycaemia Br Med J 2 272 1976

12 Editorial Beta blockers for diabetics Lancet I 843 1977

13 Eggstein M & Kreutz F H Eine neue Bestimmung der Neutralfette im Blutserum und Gewebe I Prinzip Durchführung und Besprechung der Methode Klin Wochenschr 44 262 1966

14 Garber A J Cryer P E Santiago J V Haymond M W Pagliare A J & Kipnis D M The role of adrenergic mechanisms in the substrate and hormonal response to insulin induced hypoglycaemia in man J Clin Invest 58 7 1976

15 Harms H H & van der Meer J Isoprenaline antagonism of cardioselective beta adrenergic receptor blocking agents on human and rat adipocytes Br J Clin Pharmacol 2 311 1975

16 Hedstrand H & Åberg H Insulin response to intravenous glucose during long term treatment with propranolol Acta Med Scand 196 39 1974

17 Himms Hagen J Sympathetic regulation of metabolism Pharmacol Rev 19 367 1967

18 Itaya K & Ui M Colorimetric determination of free fatty acids in biological fluids J Lipid Res 6 16 1965

19 Jacobsson B & Smith U Effect of cell size on lipolysis and antilipolytic action of insulin in human fat cells J Lipid Res 13 651 1972

20 Johnsson G Influence of metoprolol and pro-

pranolol on hemodynamic effects induced by adrenaline and physical work Acta Pharmacol Toxicol (Suppl) V 59 1975

21 Johnsson G Regårdh C G & Solvell L Combined pharmacokinetic and pharmacodynamic studies in man of the adrenergic β_1 receptor antagonist metoprolol Acta Pharmacol Toxicol (Suppl) V 31 1975

22 Kaneto A Miki E & Kosaka K Effect of beta and beta$_2$-adrenoreceptor stimulants infused intrapancreatically on glucagon and insulin secretion Endocrinology 97 1166 1975

23 Kotler M N, Besman L & Rubenstein A H Hypoglycaemia precipitated by propranolol Lancet 2 1389 1966

24 Loubatieres A Mariani M M Sorel G & Savi L The action of β adrenergic blocking and stimulating agents on insulin secretion Characterization of the type of β receptor Diabetologia 7 127 1971

25 Podolsky S & Pattavina C G Hyperosmolar non ketotic diabetic coma A complication of propranolol therapy Metabolism 22 685 1973

26 Robertson P & Porte D The glucose receptor A defective mechanism in diabetes mellitus distinct from the beta adrenergic receptor J Clin Invest 52 870 1973

27 Vedin A Wilhelmsson C & Björntorp P Induction of diabetes and oral glucose tolerance tests during and after chronic β blockade Acta Med Scand (Suppl) 575 37 1975

28 Waal Manning H J Metabolic effects of β adrenoceptor blockers Drugs (Suppl) 1 121 1976

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The Influence of Sar[1] Ala[8] Angiotensin II (Saralasin) on Plasma Aldosterone in Hypertensive Patients

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ABSTRACT *The effect of a 4 hour infusion of the* angiotensin II analogue sar[1] ala[8] angiotensin II (saralasin) on plasma aldosterone concentration (PAC) was assessed in relation to plasma renin activity (PRA) in 12 patients both on normal sodium intake and after marked sodium depletion On normal sodium intake the response of PAC to saralasin was variable following sodium depletion saralasin induced a marked decrease in PAC in 11 of 12 patients The extent of the change in PAC induced by saralasin correlated closely with log PRA The data indicate that saralasin is also a competitive antagonist of the effect of the endogenous renin–angiotensin system (RAS) on the adrenal cortex, with agonistic activity appearing at low levels of PRA The effect of sodium depletion on PAC appears to be mediated to a major degree by the RAS

Key words hypertension plasma renin activity plasma aldosterone concentration renin–angiotensin system saralasin sodium depletion

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Gradually it has become apparent that angiotensin II exerts many influences besides its pressor activity For several of these there is still discussion about the extent to which the actions have (patho)physiological relevance or are solely pharmacological effects Recently more or less specific blockers of the renin–angiotensin system (RAS) have become available Studies with these blockers have made it obvious that the RAS plays a major role in the maintenance of blood pressure (BP) in both normo- and hypertensive individuals especially during sodium restriction and ambulation (9 10 13 21)

The role of the RAS in the regulation of aldosterone secretion is also becoming clearer Several animal studies showed that angiotensin II blockade by the competitive angiotensin II antagonist *sar[1] ala[8] angiotensin II (saralasin)* induces marked decreases in aldosterone secretion stimulated by e g aortic or thoracic caval constriction or by sodium depletion (8 12 18 19) Studies on the influence of saralasin or a converting enzyme inhibitor indicate a role of the RAS in the increase in plasma aldosterone concentration (PAC) during sodium restriction in normotensive men (14 17)

As yet the effect of angiotensin II blockade on PAC of hypertensive patients before and after sodium depletion has not been evaluated This was the purpose of the present study In view of the half life of plasma aldosterone (about 20–30 min) (3) saralasin was infused for 4 hours in order to assess fully the role of the RAS in the maintenance of a given PAC

PATIENTS AND METHODS

The present results were obtained as part of a larger study on the effects of saralasin on BP and renal function in hypertensive patients The details of this study have been outlined previously (10) Combined data on plasma renin activity (PRA) and PAC were obtained in 12 patients 5 with unilateral and 3 with bilateral renal artery stenosis and 4 with essential hypertension Hypertension was characterized by standard procedures including aortography and selective renal angiography The relevant patient data are summarized in Table I

All patients were studied twice For the first study a normal sodium intake (100 mmol Na^+ daily) for at least 7 days had been used The second study was performed after sodium depletion by furosemide 40 mg twice daily orally and a daily dietary sodium intake of 20 mmol dur

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Abbreviations RAS=renin–angiotensin system PAC=plasma aldosterone concentration PRA=plasma renin activity BP=blood pressure

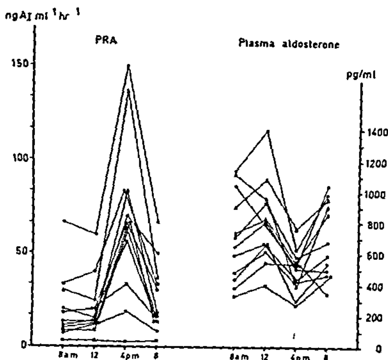


Fig 3 PRA and plasma aldosterone at 8 a m noon 4 p m and 8 p m in 12 sodium-depleted hypertensive patients Saralasin was infused from 12 to 4 p m

not increase in one patient with essential hypertension

... of saralasin after sodium depletion
... II Fig 3)

Values of PRA and PAC obtained at 8 a m noon and 8 p m again did not differ significantly from each other Infusion of saralasin during 4 hours now induced a clear increase in PRA ($p<0.01$) In contrast PAC decreased significantly ($p<0.01$) The patient with the PRA not stimulated by sodium depletion also showed an unchanged PRA and PAC during this infusion

Relationships between different parameters
(Fig 4)

A significant relationship was found between log PRA and PAC for values obtained at 8 a m $r=+0.80$ $p<0.001$ $n=24$ Also the change in log PRA induced by sodium depletion correlated with the change in PAC ($r=+0.54$ $p<0.05$ $n=12$) As shown in Fig 4 the change in PAC induced by saralasin showed a clear relationship to the initial log PRA $r=-0.84$ $p<0.001$ $n=24$ PAC correlated inversely with serum potassium $r=-0.61$ $p<0.01$ $n=24$

DISCUSSION

For the BP response to saralasin it has become clear that its antagonistic and agonistic activities upon the peripheral angiotensin II receptors are closely related to the level of endogenous PRA (6 10) Regarding the effect of saralasin on plasma aldosterone concentration Hollenberg et al (9) and Noth et al (14) showed an agonistic action in normotensive individuals on a normal sodium intake and a small antagonistic action following short term (60 min) saralasin infusion after 3 days of sodium restriction (14) In the present study the effect of prolonged (4 h) infusion of saralasin on PAC was assessed in hypertensive patients before and after marked sodium restriction The results show a close relationship between log PRA and PAC as well as between the change in PAC induced by saralasin and the initial log PRA Below a PRA level of 2–4 ng A I ml⁻¹h⁻¹ a level observed in normal subjects (10) the agonistic activity of saralasin on aldosterone secretion was apparent above this level the antagonistic activity prevailed A similar relationship was found between the change in BP induced by saralasin and the initial log PRA in hypertensive patients (10) These data suggest therefore that both the BP and the aldosterone secretion become dependent on the RAS to a de

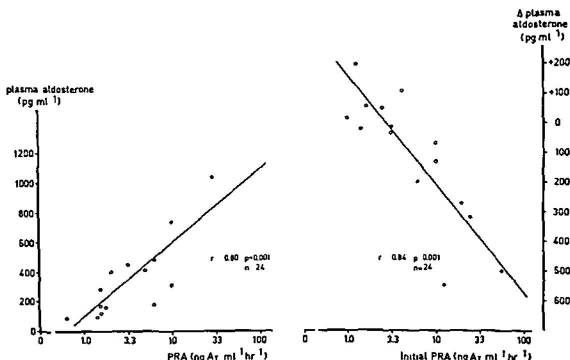


Fig 4 Relationship between log PRA and plasma aldosterone and between log initial PRA and change in plasma aldosterone induced by saralasin in hypertensive patients before (O) and after (●) sodium depletion

gree closely related to the increase in the level of the PRA at least in hypertensive patients with or without sodium depletion As far as can be con cluded from these experiments with saralasin the threshold for renin dependency appears to be the same for BP and aldosterone The present experi ments do not allow conclusions regarding changes in receptor sensitivity caused by sodium depletion

There is still doubt regarding the precise role of the RAS in the increase in aldosterone secretion following sodium depletion (1 20) However the present data in hypertensives as well as the results in normotensive people with blockade of the RAS by saralasin or a converting enzyme inhibitor (17) clearly indicate that the RAS is the most important mediator of the increase in aldosterone secretion following sodium depletion This conclusion does not exclude of course that following blockade of the RAS sodium depletion can induce an increase in aldosterone secretion via other mechanisms such as changes in ACTH or in intracellular sodium or potassium (11 15)

Following angiotensin II blockade by saralasin PAC remained significantly ($p<0.01$) above the concentration observed on normal sodium intake This could suggest that this part of the increase in PAC following sodium depletion is not related to the RAS Changes in the metabolic clearance rate of aldosterone as well as other stimuli could be im plicated in this part of the increase in PAC How ever also this part could be caused by the RAS this part could be mediated by the recently proposed specific adrenal cortical receptors for the hep-tapeptide [des asp[1]] angiotensin II which seem to be less easily blocked by angiotensin II antagonists (2 4 5)

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REFERENCES

1 Best J B Coghlan J P Bett J H N & Cran E J Circulating angiotensin II and aldosterone levels during dietary sodium restriction Lancet 2 1353 1971
2 Blair West J R Coghlan J P Denton D A Funder J W Scoggins B A & Wright R D The effect of the heptaude (2–8) and the hexapeptide (3–8)

fragments of angiotensin II on aldosterone secretion J Clin Endocrinol Metab 32 575 1971
3 Bojesen E Aldosterone in peripheral plasma of normal man In Aldosterone (ed E E Baulieu & P Robel) p 163 Blackwell Scientific Publications Oxford 1964
4 Bravo E L Khosla M C & Bumpus F M New aspects of aldosterone regulation Mayo Clin Proc 52 308 1977
5 Campbell W B Brooks S N & Pettinger W A Angiotensin II and angiotensin III induced aldosterone release in vivo in the rat Science 184 994 1974
6 Case D B Wallace J M Keim H J Sealey J E & Laragh J H Usefulness and limitations of saralasin a partial competitive agonist of angiotensin II for evaluation of the renin and sodium factors in hypertensive patients Am J Med 60 825 1976
7 Freedlender A E Fyhrquist F & Hollemans H J G In Methods of hormone radio immunoassay (ed B M Jaffe & H R Behrman) p 455 Academic Press New York and London 1974
8 Freeman R H Davis J O & Spielman W S Renin–angiotensin system and aldosterone secretion during aortic constriction in the rat Am J Physiol 232 F434 1977
9 Hollenberg N K Williams G H Burger B Ishiwaka I & Adams D F Blockade and stimulation of renal adrenal and vascular angiotensin II receptors with 1 sar 8 ala angiotensin II in normal man J Clin Invest 57 39 1976
10 van Hoogdalem P Donker A J M & Leenen F H H Angiotensin II blockade before and after marked sodium depletion in patients with hypertension Clin Sci Mol Med 54 75 1978
11 Laragh J H Potassium angiotensin and the dual control of aldosterone secretion N Engl J Med 289 745 1973
12 Lohmeier Th E Davis J O Hanson R C & Williams G M Renin–angiotensin–aldosterone system in rabbits with thoracic caval constriction Am J Physiol 232 F559 1977
13 MacGregor G A & Dawes P M Agonist and antagonist effects of sar 1 ala 8-angiotensin II in salt loaded and salt-depleted normal man Br J Clin Pharmacol 3 483 1976
14 Noth R H Tan S Y & Mulrow P J Effects of angiotensin II blockade by saralasin in normal man J Clin Endocrinol Metab 45 10 1977
15 Oparil S & Haber, E The renin–angiotensin system (first of two parts) N Engl J Med 291 389 1974
16 Pratt J J Boonman R Woldring M G & Donker A J M Special problems in the radioimmunoassay of plasma aldosterone without prior extraction and purification Clin Chim Acta 84 329 1978
17 Sancho J Re R Burton J Barger A C & Haber E The role of the renin–angiotensin–aldosterone system in cardiovascular homeostasis in normal human subjects Circulation 53 400 1976
18 Spielman W S & Davis J O The renin angiotensin system and aldosterone secretion during sodium depletion in the rat Circ Res 35 615, 1974
19 Stephens G A Davis J O Freeman R H Watkins B E & Khosla M C The effects of angiotensin II blockade in conscious sodium depleted dogs Endocrinology 101 378 1977
20 Williams G H Bailey G L Hampers C L Lauler D P Merill J P Underwood R H Blair West J R Coghlan J P Denton D A Scoggins B A & Wright R D Studies on the metabolism of aldosterone in chronic renal failure and anephric man Kidney Int 4 280 1973
21 Williams G H & Hollenberg, N K 1 Sar 8 ala angiotensin II s effect on renal adrenal and vascular receptors in man In Systemic effects of antihypertensive agents (ed M P Sambhi) p 531 Stratton New York 1976

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Minoxidil in Refractory Hypertension

Effects on Blood Pressure Plasma Volume and Muscle Blood Flow

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ABSTRACT Ten patients, refractory to antihypertensive treatment with diuretics, β adrenergic blocking drugs and hydralazine, were investigated They had previously shown increased total peripheral resistance as well as increased blood flow resistance at rest and at maximal vasodilatation in a local vascular bed (calf) compared to hypertensive patients responding adequately to the same triple drug treatment The results showed no differences between the groups in vascular smooth muscle tone at rest or in plasma volume It was concluded that the inadequate response to therapy was mainly caused by a vascular abnormality interpreted as structural adaptive changes of the arteriolar vessel wall, more pronounced in the patients refractory to treatment The patients were then treated with minoxidil (15–35 mg/day) as the only vasodilating agent, the dose of the β adrenergic blocking agent and the diuretic therapy being kept almost constant Plasma volume (Evans Blue) measurements and peripheral hemodynamic investigations were made during the previous regimen and when an acceptable BP reduction had been achieved on the combined treatment with minoxidil Peripheral hemodynamic experiments on the calf blood flow were made with venous occlusion plethysmography at rest (resting flow and resistance) and after arterial occlusion and muslce work (maximal flow and resistance at maximal dilatation) BP was recorded simultaneously with the flow determinations indirectly in the right arm After the change to minoxidil all patients displayed a reduction of arterial BP and reached adequate levels within two months This BP reduction correlated positively to an increase in resting blood flow and was associated with decreased peripheral resistance at rest A tendency towards increased plasma volume was demonstrated No change was found in the resistance at maximal dilatation Consequently, no sign of reversibility of the vascular abnormality was demonstrated after 1–2 months of BP lowering therapy

Key words hypertension refractory minoxidil hydralazine vascular hypertrophy

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The hemodynamic pattern in established hypertension is characterized by an increased total peripheral resistance (8 9 18 22) Evidence is now supporting the view that this increase in vascular resistance is caused by an adaptive thickening of the arteriolar wall (5 7 10 23 25) This vascular abnormality is probably also causing the change in baroreceptor response in hypertension (1 24) Furthermore the concept of an increased arteriolar wall/lumen ratio explains satisfactorily the increased reactivity observed in hypertensive patients (7 23)

In a recent study (2) total peripheral resistance as well as blood flow resistance during maximal vasodilatation in two vascular beds (hand and calf) were found to be considerably increased in refractory hypertension when compared to hypertensive patients responding adequately to the same therapy i e combined treatment with diuretics propranolol and hydralazine In fact the peripheral resistance was the main factor responsible for the refractory state since cardiac output and plasma volume did not differ between the groups However also the resistance vessels of these refractory patients displayed the capacity for considerable vasodilatation caused by the potent stimulus of ischemia This led to the assumption that treatment of these refractory patients might be successful if they had a sufficiently potent vasodilating drug

Minoxidil is a vasodilating drug that acts directly on the arteriolar smooth muscle (6 13 21) It has sodium retaining properties (14) and like all vaso

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Abbreviations BP=blood pressure MAP=mean arterial BP HR=heart rate

Table I *Age weight glomerular filtration rate (GRF) and heart volume (X ray) of the patients studied*

Pat no	Age (y)	Weight (kg)	GFR (ml/min m²)	Heart volume (ml/m²)
1	54	105	89	610
2	52	90	74	500
3	59	100	89	440
4	52	90	82	590
5	60	79	74	600
6	57	95	72	470
7	57	83	80	470
8	58	92	82	410
9	57	88	86	460
10	50	98	79	600
Mean	55.6	92	81	515
S D	3.4	7.8	6.1	76.8

dilating compounds will increase cardiac output because of baroreceptor reflex activity (13, 14)

The present study was performed in ten patients refractory to triple drug antihypertensive therapy (diuretics propranolol and hydralazine) The vasodilating agent was changed to minoxidil, and the effect on blood pressure (BP) and plasma volume as well as peripheral blood flow and resistance during rest and during maximal flow conditions was compared with the effect of the previous therapy

PATIENTS

Ten hypertensive patients all middle aged men were checked for drug defaulting using pill count and riboflavin tablets with urinary examination (2) All patients were shown to have a high degree of β-adrenergic blockade using isoproterenol infusion tests and their acetylation phenotype was determined with isoniazid plasma concentration curves (2) They were all slow acetylators and had been investigated in accordance with our routines (3) Furthermore a renal arteriography was performed and the glomerular filtration rate (Cr^{51} EDTA) was determined (11) in all patients No patient showed evidence of secondary forms of hypertension They all had grade II eye ground changes The patient data are given in Table I

The patients were treated initially with diuretics either hydrochlorothiazide 50 mg daily or bendroflumethiazide 5 mg daily and propranolol 120–160 mg t i d The vasodilating therapy consisted of hydralazine 50 mg t i d in all but three patients (nos 1 3 and 10) who had 75 mg t i d All ten patients had also had additional treatment with either spironolactone furosemide or bethanidine with unsatisfactory BP reducing effect or side-effects This treatment had been discontinued one month before the hemodynamic investigations On the triple drug therapy described none of the patients had a reduction of mean arterial pressure (MAP) of 10% or more Neither were their BPs below 200/110 mmHg at the clinic

METHODS

The dose of the β adrenergic blocking drug was kept constant while the hydralazine treatment was discontinued and replaced by minoxidil Concerning the diuretic therapy this too was kept constant in all but one patient (no 7) who additionally received furosemide 40 mg daily because of ankle edema The initial minoxidil dose was 2.5 mg b i d and this was increased with 5 mg/day at weekly intervals until satisfactory BP reduction was achieved During the first two months the patients were examined every week concerning BP heart rate (HR) weight and side effects Thereafter these examinations were made every month S-electrolytes and S creatinine were followed every month Plasma volume was estimated and peripheral hemodynamic investigations were performed during the previous triple drug therapy and when satisfactory BP control had been achieved after 1–2 months with minoxidil in the combined treatment

The drugs used were provided by the Upjohn Company Kalamazoo Mich USA through the Swedish subsidiary Upjohn AB Partille

BP was measured at the Out Patient Clinic by one and the same nurse with a mercury manometer and stethoscope The rubber balloon was 12 cm wide and 26 cm long The measurements were performed on the right arm in the supine position and recorded to the nearest 2 mmHg The diastolic BP was determined at phase 5 (disappearance) HR was determined by palpation of the radial pulse immediately before the BP measurement Basal BP was measured in the morning at the laboratory after 30 min supine rest in a quiet room The same device and technique were used but another nurse made the recordings MAP was calculated as the diastolic BP plus 1/3 of the pulse pressure

Plasma volume was determined with Evans Blue in the morning after 40 min supine rest Blood samples were drawn 5 10 and 20 min after the injection The hypothetical plasma concentration of the dye at zero time was estimated from a plot of log concentration against time From that concentration and the originally injected amount of dye plasma volume was calculated (16) and expressed in absolute figures and in ml/cm body height (4 26)

The peripheral blood flow experiments before and during minoxidil treatment were performed in the morning before the patients had taken their morning doses of antihypertensive drugs Before the first investigation the arterial circulation to the legs was examined by oscillometry (12) to exclude arteriosclerotic or other causes of impaired large vessel blood flow The blood flow to the calf muscle vascular bed was determined by venous occlusion plethysmography using the strain gauge technique (27) BP was measured simultaneously with the flow determinations indirectly in the right arm and diastolic BP was determined at phase 5 After 30 min supine rest five flow determinations were made The flow resistance un-

Table II *Auscultatory MAP (mmHg) and HR (beats/min) at the clinic before treatment and during triple drug therapy with hydralazine or minoxidil as the vasodilating drug*

Pat no	Pretreatment		Triple therapy Hydralazine		Minoxidil	
	MAP	HR	MAP	HR	MAP	HR
1	153	74	144	68	105	74
2	156	80	150	53	102	59
3	153	90	145	60	120	64
4	162	76	149	72	117	70
5	153	97	148	63	113	68
6	160	84	145	75	110	74
7	145	76	150	68	116	72
8	147	56	135	55	124	60
9	155	68	140	58	115	56
10	143	84	144	67	122	70
Mean	153	79	145	64	114	67
S D	6 2	11 5	4 7	7 3	7 1	6 53
p			<0 01	<0 01	<0 001	<0 01
					p<0 001	ns

der these circumstances was designated "*resting resistance*" Then arterial occlusion was applied during 4–5 min and the patient exercised the calf muscles by pedalling on a special foot-ergonometer until ischemic pain The pedalling time was measured Then the arterial occlusion was released and 3–5 readings of blood flow followed The flow resistance after this procedure was designated "*resistance at maximal dilatation*" or "*minimal resistance*" The ratio between flow resistance at rest and that during maximal vasodilatation is referred to in the following as "*resting tone*" as it is considered to reflect the extent of smooth muscle concentration in the resistance vessels of the calf muscle during rest (7 23) Blood flow resistance is

Table III *Basal auscultatory MAP (mmHg) during triple drug therapy with hydralazine (I) and minoxidil (II)*

Pat no	MAP I	MAP II
1	134	110
2	166	125
3	126	111
4	133	99
5	136	131
6	114	110
7	138	110
8	124	116
9	116	98
10	134	118
Mean	132	113
S D	14 5	10 3
p	<0 01	

expressed in PRU_{100} i e mean BP (mmHg) divided by blood flow (ml/100 ml min)

Standard methods were used for the calculation of the mean (x) the standard deviation (S D) and the linear correlation coefficient (r) The hypothesis of no difference in means was tested with t test for paired observations Only two-tailed tests were used and differences were considered significant for p values of 0 05 or less

RESULTS

Blood pressure

After 4–8 weeks on increasing doses of minoxidil all patients had a BP below 170/100 mmHg i e what is considered to be an adequate BP reduction at the clinic The reduction of MAP from pretreatment values was 39 mmHg (p<0 001) while from combined treatment with hydralazine it was 31 mm (p<0 001) (Table II) The average dose of minoxidil was 19 mg/day (range 15–35) The reduction of MAP during basal conditions was also significant—19 mmHg (p<0 01) (Table III)

Initially when the patients were on hydralazine the MAPs at the clinic were significantly higher than during basal conditions When they were on combined treatment with minoxidil no difference was found between BP at the clinic and basal BP Significant correlations were found for BP during basal conditions and BP at rest during the flow experiments when the patients were on hydralazine r=0 75 p<0 05 and when on minoxidil r=0 93 p<0 001

Table IV *Plasma volume before (B) and during (D) minoxidil treatment*

Pat no	Total plasma volume (l)		Plasma volume per body height (ml/cm)	
	B	D	B	D
1	3 7	4 1	21 4	23 7
2	3 1	3 6	17 8	20 7
3	3 1	3 6	18 0	20 9
4	4 8	3 9	26 8	21 8
5	2 6	3 4	14 8	19 4
6	3 6	4 5	20 7	25 9
7	3 6	4 0	20 2	22 5
8	3 5	3 6	19 6	20 1
9	3 4	3 5	19 2	19 8
10	3 3	3 3	19 0	19 0
Mean	3 47	3 75	19 8	21 4
S D	0 57	0 36	3 09	2 15
p	ns		ns	

Plasma volume

The hematocrits did not differ between the two examinations and consequently differences in total blood volume are reflected by the plasma volume (Table IV) There were increases though not quite significant in plasma volume and plasma volume [illegible] cm body height when minoxidil was given [illegible] no correlation was found between the change in BP and the changes in total plasma volume and plasma volume per cm body height

Peripheral hemodynamic investigation

The results from the blood flow measurements in the calf are shown in Table V The average blood flow at rest was significantly increased when the patients were on minoxidil treatment ($p<0\ 001$) and since the BP was decreased so was the calculated resistance at rest ($p<0\ 01$) After arterial occlusion and muscle work the blood flow increased to the same extent before and during minoxidil Resistance at maximal dilatation did not change when treatment was changed from hydralazine to minoxidil There was a significant correlation between BP reduction during basal conditions and change in resting blood flow ($r=0\ 66$ $p<0\ 05$) (Fig 1), whereas no correlation was found between BP *reduction and change in resting resistance* A significant reduction of resting tone was demonstrated when the patients were on minoxidil (Table V)

Side effects

One patient (no 7) developed mild ankle edema when receiving minoxidil 20 mg daily This sign disappeared when furosemide 40 mg daily was added to the triple drug treatment Eight patients had mild hypertrichosis of the face trunk and arms No patient showed significant weight gain or complained of symptoms of any kind No abnormal changes from pretreatment levels of S creatinine or S-electrolytes were observed No tachycardia was

Table V *Resting blood flow and resting resistance (R_{rest}) blood flow and resistance at maximal dilatation (R_{min}) and resting tone before (B) and during (D) minoxidil treatment*

Pat no	Resting flow (ml/100 ml min)		R_{rest} (PRU_{100})		Maximal flow (ml/100 ml min)		R_{min} (PRU_{100})		Resting tone (R_{rest}/R_{min})	
	B	D	B	D	B	D	B	D	B	D
1	2 8	4 1	47 3	28 0	33 3	31 0	4 6	4 2	10 3	6 7
2	3 1	4 9	44 9	24 3	41 6	34 6	3 3	3 7	13 6	6 6
3	2 7	4 7	45 0	23 6	39 0	46 3	4 1	2 5	11 0	9 4
4	3 0	5 6	39 4	17 9	37 1	46 3	3 7	2 6	10 6	6 9
5	1 6	2 6	81 3	51 5	27 7	25 0	5 0	5 7	16 3	9 0
6	2 4	3 4	52 1	32 9	52 2	40 0	2 4	2 8	19 3	11 8
7	3 2	5 2	40 8	21 2	44 8	39 0	3 1	4 5	13 2	4 7
8	2 5	3 6	46 8	34 7	39 5	33 0	3 1	3 7	15 1	9 4
9	1 8	2 4	65 2	41 3	41 9	38 1	3 0	2 8	21 7	14 8
10	2 4	3 5	50 1	34 9	32 4	30 7	3 5	3 0	14 3	11 6
Mean	2 6	4 0	51 3	31 03	39 0	36 4	3 58	3 55	14 5	9 1
S D	0 52	1 08	12 75	10 19	6 90	6 87	0 79	1 02	3 7	3 0
p	<0 001		<0 001		ns		ns		<0 001	

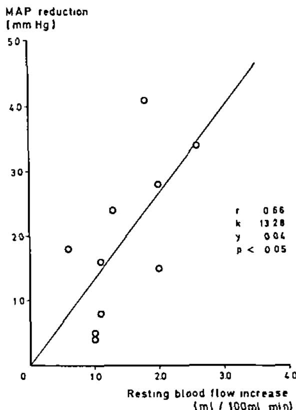


Fig 1 Correlation between basal mean BP reduction and increase in resting blood flow while on minoxidil treatment

noted (Table II) No patient wanted the drug to be discontinued when offered this option The observation period is at present one year

DISCUSSION

Patients responding unsatisfactorily to antihypertensive treatment are not uncommon although the usual reason is failure to comply or insufficient drug regimens However when drug defaulters are excluded and potent combined drug therapy is used a minor number of patients with primary hypertension still show poor BP control (2) In such cases with an increased risk of complications it must be considered justified to use relatively untested hypertensive agents in order to establish adequate BP control Although cardiotoxic effects in dogs have been described (6) it was considered appropriate to use mixoxidil in the present patients especially as an increased vascular resistance was their general hemodynamic pattern Furthermore the components of a suitable combined therapy—diuretics and β adrenergic blockers—were already instituted and tolerated

Arterial occlusion and superimposed muscular work are shown to produce powerful vasodilatation in the forearm and hand (7 23) Arterial occlusion during 5 min produces a hyperemia that is not overcome by intravenous infusion of norepinephrine nor can intra arterial infusions of adenosine triphosphate increase the maximal blood flow induced by ischemia (5) A procedure for maximal vasodilatation in the calf has not been described previously However preliminary data indicate that arterial occlusion and simultaneous muscle work produce an almost complete relaxation of the vascular smooth muscles in this vascular bed Thus intra arterial infusions of large doses of isoproterenol (3 μg/min) in the femoral artery did not add further to the dilatation induced by ischemic work Furthermore the vasoconstriction expected from large doses of norepinephrine (3 μg/min) was almost overridden by ischemic work To test the variability of maximal flow determinations after ischemic work repeated measurements were made in 20 patients (2) The variation coefficient for these consecutive determinations was 9 1%

The calculation of blood flow resistance in the calf was based on auscultatory arm BP and plethysmographic blood flow determinations It might be questioned whether the indirect BP recordings in the arm are representative for the perfusion pressure in the calf during maximal blood flow However simultaneously measured intra arterial BP in the brachial and femoral arteries during maximal vasodilatation was found to correspond closely in a model experiment (2) Auscultatory arm BPs measured simultaneously in the same model experiment showed less agreement initially during 5–10 sec when a rapid BP reduction took place but corresponded well during resting conditions and when measured more than 10–15 sec after the arterial occlusion had been released The variation coefficient for three consecutive determinations in 20 patients was 9 7%

All patients in the present study had a considerable BP reduction when minoxidil was instituted (Tables II and III) As they were previously shown to have a high degree of β adrenergic receptor blockade it was not surprising that the HR did not increase However the possibility that stroke volume increased cannot be ruled out Otherv

altered blood flow distribution was achieved, since blood flow to the calf muscles was increased about 50% during rest

A considerable difference between casual BP and BP after increasing periods of rest is well known In that sense it is remarkable that our patients had a convincing reduction of basal BP compared to BP at the clinic while on hydralazine but no such reduction during minoxidil treatment (Tables II and III) One explanation for this observation might be systemic differences during the measurements of basal BP giving unduly high results when the patients were receiving minoxidil This is unlikely however since the method was carefully standardized Another possibility is that the patients during minoxidil treatment when they were followed more frequently than during the previous regimen were more relaxed during the BP measurements at the clinic This is conceivable yet all ten patients had been followed at the clinic for several years and being refractory to therapy had been examined frequently The two factors might interact but the effect of minoxidil treatment on BP is still convincing since a reduction was demonstrated both at the clinic and during quiet rest in the laboratory A theoretical explanation for reduced BP at the clinic compared to basal conditions is that the patients during minoxidil therapy might be less sensitive to mild stress vasoconstriction because of the intense vasodilatation induced by minoxidil

One important question was whether the patients would retain sodium and water during minoxidil therapy However all patients except one (no 7) who developed ankle edema remained on their initial dose of diuretics throughout the study No weight gain was observed and only a discrete increase in plasma volume (Table IV) Consequently the thiazide diuretic therapy was sufficient to serve as the diuretic balance in the triple drug combination It must be remembered however that all patients had a normal renal function (Table I) and that a more potent diuretic therapy might be needed in patients with impaired renal function (17 20)

The results from the peripheral blood flow experiments clearly show a superior vasodilating potency of minoxidil compared to hydralazine (Table V) During resting conditions the blood flow was increased and the BP decreased, reflecting a highly significant reduction of flow resistance Furthermore resting tone, considered as the actual extent of smooth muscle contractation of the resistance vessels during rest was decreased during minoxidil therapy However no increase was observed in resistance at maximal flow This fact led us to conclude that the vascular abnormality in our patients refractory to treatment did not show any tendency to be reversible as has previously been demonstrated in animal studies (19) as well as in man (15) It must be emphasized however that the observation time probably is too short for such changes to be expected

In summary the ten patients refractory to triple drug treatment showed an adequate BP reduction when treated with minoxidil instead of hydralazine This BP reduction was caused by a vasodilatation resulting in a decreased vascular resistance and increased blood flow in the calf muscular vessels at rest Conventional diuretic drugs in small doses and β adrenergic blocking therapy proved to be suitable components in the combined therapy There was no tendency however towards reduced resistance at maximal vasodilatation and consequently the vascular abnormality remained unaltered at least shortly after a normal BP level had been achieved Patient acceptance of the drug was excellent and minoxidil should be regarded as an alternative in the combination therapy of refractory hypertension

REFERENCES

1 Aars H Relationship between aortic diameter and aortic baroreceptor activity in normal and hypertensive rabbits Acta Physiol Scand 75 406 1969

2 Andersson O Management of hypertension Clinical and hemodynamic studies with special reference to patients refractory to treatment Acta Med Scand (Suppl) 617 1978

3 Andersson O Berglund G Hansson L Sannerstedt R Sivertsson R Wikstrand J & Wilhelmsen L Organization and efficacy of an out patient hypertension clinic Acta Med Scand 203 301 1978

4 Chien S Usami S Simmons R L McAllister F F & Gregersen M I Blood volume and age repeated measurements on normal men after 17 years J Appl Physiol 21 583 1966

5 Conway J A vascular abnormality in hypertension A study of blood flow in the forearm Circulation 27 520 1963

6 DuCharme D W Freyburger W A Graham B E & Carlson R G Pharmacologic properties of minoxidil a new hypotensive agent J Pharmacol Exp Ther 184 662 1973

7 Folkow B Grimby G & Thulesius O Adaptive structural changes of the vascular walls in hypertension and their relation to the control of the peripheral resistance Acta Physiol Scand 44 255 1958

8 Fries E D Hemodynamics of hypertension Physiol Rev 40 27 1960
9 Frohlich E D Ulrych M Tarazi R C Dustan H P & Page I H A hemodynamic comparison of essential and renovascular hypertension Cardiac output and total peripheral resistance in supine and tilted Circulation 35 289 1967
10 Furuyama M Histometrical investigations of arteries in reference to arterial hypertension Tohoku J Exp Med 76 388 1962
11 Garnett E S Parsons V & Veau N Measurement of glomerular filtration rate in man using ^{51}Cr/Edatic Acid complex Lancet 1 818 1967
12 Gesenius H Oscillographie und Arteriographie Dtsch Med Wochenschr 1 1 1949
13 Gilmore E Weil J & Chidsey C Treatment of essential hypertension with a new vasodilator in combination with beta adrenergic blockade N Engl J Med 282 521 1970
14 Gottlieb T B Katz, F H & Chidsey C A III Combined therapy with vasodilator drugs and beta adrenergic blockade in hypertension A comparative study of minoxidil and hydralazine Circulation 45 571 1972
15 Hansson L & Sivertsson R Effect of blood pressure reduction on the structural vascular abnormality in skin and muscle vascular beds in human essential hypertension Clin Sci Mol Med 51 77 1976
16 Lawson H C Handbook of physiology sect 2 vol 1 p 23 American Physiological Society Washington 1962
17 Limas C J & Freis E D Minoxidil in severe hypertension with renal failure Effects on its addition to conventional antihypertensive drugs Am J Cardiol 31 355 1973
18 Lund Johansen P Hemodynamics in early essential hypertension Acta Med Scand (Suppl) 482 1967
19 Lundgren Y Regression of structural cardiovascular changes after reversal of experimental renal hypertension in rats Acta Physiol Scand 91 275 1974
20 Pettinger W A & Mitchell H C Minoxidil—an alternative to nephrectomy for refractory hypertension N Engl J Med 289 167 1973
21 Pluss R G Orcutt J & Chidsey A Tissue distribution and hypotensive effects of minoxidil in normotensive rats J Lab Clin Med 79 639 1972
22 Sannerstedt R. Hemodynamic response to exercise in patients with arterial hypertension Acta Med Scand (Suppl) 458 1966
23 Sivertsson R The hemodynamic importance of structural vascular changes in essential hypertension Acta Physiol Scand (Suppl) 343 6 1970
24 Sleight P Baro-receptor function in hypertension In Pathophysiology and management of arterial hypertension (ed G Berglund L Hansson & L Werko) pp 45–53 Lindgren & Soner Molndal 1975
25 Suwa N & Takahashi T Morphological and morphometrical analysis of circulation in hypertension and ischaemic kidney Urban & Schwarzenberg Munchen Berlin and Wien 1971
26 Tarazi R C Dustan H P Frohlich E D Gifford R W & Hoffman G C Plasma volume and chronic hypertension relationship to arterial pressure levels in different hypertensive diseases Arch Intern Med 125 835 1970
27 Whitney R J The measurement of volume changes in human limbs J Physiol 121 1 1953

Insulin in Portal, Hepatic and Peripheral Venous Blood after Glucose, Tolbutamide and Glipizide Stimulation

I d cat on of Insi l n Release from Per pheral Tissues

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ABSTRACT Insul n in portal hepatic and/or peripheral venous blood was determined in 16 patients admitted to a surgical ward for various diseases Portal venous blood was obtained via a catheter introduced into the portal vein either through the umb l cal vein remnant or transhepatically Four subjects were given a peroral load of glucose followed after 60 min by i v tolbutamide In simultaneous blood samples two of these subjects showed higher insulin concentrations in peripheral venous blood than in portal venous blood Twelve subjects were given i v glipizide In one subject blood samples were drawn from the portal vein a hepatic vein and a peripheral vein in five subjects from the portal vein and a peripheral vein and in six subjects from the portal vein and a hepatic vein Two subjects showed h gher insulin concentrations in peripheral venous blood than in portal venous blood The mean peripheral insulin response (six subjects) was of the same magnitude as the mean hepatic insulin response (six subjects) It is suggested that these findings reflect a release of previously bound insulin from peripheral tissues

Key wo ds nsul n portal hepa c and peripheral venous blood n man

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To determine how well peripheral venous insul n levels reflect those n the portal ve n portal and peripheral blood samples obtained simultaneously have been stud ed (1 2 4 5 8) The results indicate that wh le the courses of the insul n changes in portal and peripheral venous blood generally follow each other s gn ficant quant tative d fferences do occur between pat ents The reason is not clear Probably there are d fferences in the hepat c and/or peripheral extract on of insul n In order to study this we have assayed insul n levels in blood samples obtained s multaneously from portal hepatic and peripheral veins The samples were drawn ... and after β cell stimulation with peroral ... i v tolbutam de and i v glip z de

PATIENTS

The pat ents were 10 women and ... Surgical Department for var ous abdo ... (Table I) All were non-obese

METHODS

Blood glucose was determ ned enzym ... ox dase method us ng comme c al reage ... Stockholm Sweden) Imn uno e ... was assayed by a double an body p ocedu ... described by Soeldner and Slone (13)

One or two days befo e the s udy ... duced nto the portal ve n—f ... —e ther through the umb l cal ... hepat cally (14) Portography was ... of the patients showed ev dence of ... After th s exam na on a slow d ... solut on (1 000 IU hepar n/1 000) ... tal catheters patent The po tal ... patients any essent al d ... about the ward and eat ... morn ng on the day of he study ... percutaneously nto a femoral ... fluo oscop c control to ... 1 11) A check was al o made ... the portal catheter la d n ... stud es were perf rmed ... hours) w th the pat ent ... patients (nos 1–4) a pe ... an v nject on of to b ... Hoechst) by 60 m n In ... drawn s multaneously ... n ve ns In 12 pa ents ... al Carlo Erba) was g ... ts samples were d awn ... tic ve ns n s x of ... n hepatic and periphe ... er s multaneously ... ak remaining ... ts

Table I *Vital data laboratory data and diagnosis*

Pat no	Sex	Age (y)	Bilirubin (μmol/l)	Alk phosph (μkat/l)	Diagnosis
1	♀	50	18	5	Hodgkin s disease
2	♂	70	15	32	Carcinoma of the papilla Vateri
3	♂	61	200	42	Hepatic cirrhosis
4	♂	40	12	4 5	Abdominal observation
5	♂	65	9	2 7	Chronic pancreatitis
6	♂	63	140	8 4	Pancreatic cancer
7	♀	74	14	3	Pancreatic cancer
8	♀	41	12	3	Chronic pancreatitis
9	♀	59	106	18	Chronic pancreatitis
10	♀	65	8	10	Primary liver carcinoma
11	♀	65	4	9 8	Benign stenosis of the common bile duct
12	♀	58	60	10	Carcinoma of the papilla Vateri
13	♀	52	15	7	Duodenal carcinoma
14	♀	60	79	31	Carcinoma of caput pancreatis
15	♂	45	14	3	Chronic pancreatitis
16	♀	37	4	9 8	Benign stenosis of the common bile duct
Normal values			<20	<4 5	

Table II *Glucose (mM/l) before and after β-cell stimulation*

PoGC=portal glucose concentration HGC=hepatic venous glucose concentration PeGC=peripheral venous glucose concentration

		Oral glucose+i v tolbutamide							
Pat no		0′	30	60	62′	67	70	90	
1	PoGC	4 1	9 8	10 5	10 5	10 8	10 9	11 5	
	HGC	4 9	9 5	10 5	10 9	10 5	11 0	11 4	
	PeGC	4 1	8 0	8 7	9 0	9 3	9 6	9 9	
2	PoGC	4 9	11 8	15 5	17 0	17 0	16 9	15 6	
	HGC	5 3	11 3	15 0	15 7	16 2	16 3	16 3	
	PeGC	4 6	9 4	11 9	12 7	13 2	14 2	14 8	
3	PoGC	4 9	8 0	9 0	9 7	9 6	10 1	9 3	
	HGC	5 3	8 3	9 0	9 6	9 5	9 2	9 2	
	PeGC	4 8	6 6	7 7	7 4	7 2	7 7	7 2	
4	PoGC	4 6	7 8	10 0	9 3	9 8	8 8	7 1	
	HGC	5 1	7 5	9 1	9 4	9 4	8 2	6 7	
	PeGC	4 6	6 5	7 9	7 4	6 9	6 9	5 3	
		I v glipizide							
		0	2	5	10	20	30	45	60
5–11	PoGC	5 7	5 5	5 6	5 4	5 0	4 9	4 8	3 9
	(M±S E)	±0 5	±0 5	±0 7	±0 6	±0 6	±0 6	±0 4	±0 4
	HGC	6 1	6 1	6 0	6 3	5 2	5 0	5 0	4 2
	(M±S E)	±0 5	±0 5	±0 6	±0 4	±0 6	±0 7	±0 4	±0 4
11–16	PoGC	4 8	4 6	4 7	4 6	4 2	3 7	3 2	3 2
	(M±S E)	±0 1	±0 1	±0 1	±0 1	±0 1	±0 1	±0 2	±0 1
	PeGC	4 6	4 7	4 6	4 5	4 1	3 8	3 0	3 0
	(M±S E)	±0 2	±0 1	±0 1	±0 2	±0 2	±0 2	±0 2	±0 1

Table III *Insulin (mU/l) before and after β-cell stimulation*

PoIC±portal insulin concentration HIC=hepatic venous insulin concentration PeIC=peripheral venous insulin concentration

Pat no		Oral glucose+i v tolbutamide							
		0	30	60	62	67	70	90	
1	PoIC	31	108	150	220	204	320	215	
	HIC	10	74	142	185	132	185	192	
	PeIC	6	39	74	142	81	177	106	
2	PoIC	27	48	46	104	107	98	154	
	HIC	7	15	27	58	83	71	68	
	PeIC	13	44	38	122	81	74	132	
3	PoIC	2	40	43	181	151	127	173	
	HIC	1	17	27	51	55	64	63	
	PeIC	2	6	13	93	28	32	42	
4	PoIC	29	112	217	345	380	530	380	
	HIC	5	36	54	245	250	290	320	
	PeIC	16	73	171	310	505	670	310	
		I v glipizide							
		0	2	5	10	20	30	45	60
5–10	PoIC	36	217	183	171	89	86	80	50
	(M±S E)	±6	±43	±37	±31	±28	±19	±13	±5
	HIC	23	60	60	51	38	33	27	28
	(M±S E)	±5	±24	±24	±12	±8	±8	±7	±6
11–16	PoIC	16	105	104	106	96	74	44	39
	(M±S E)	±5	±20	±26	±22	±25	±21	±14	±12
	PeIC	11	29	73	65	46	36	24	19
	(M±S E)	±1	±4	±19	±13	±6	±15	±3	±2

Insulin concentrations presented as mean ±S E M were compared using Student s *t* test

RESULTS

Blood glucose In patients 1–4 fasting blood glucose was highest in hepatic venous blood and did not differ essentially between peripheral and portal venous blood (Table II) After glucose administration blood glucose increased in all the vessels sampled for blood The highest level was found in portal venous blood lower in hepatic venous blood and lowest in peripheral venous blood In patients 5–16 i v glipizide resulted in a decrease in blood glucose of the same magnitude in portal hepatic and peripheral venous blood (J 6–1 9 mM/l Table II) Blood glucose in hepatic venous blood exceeded that in portal venous blood (patients 5–11) Blood glucose in portal venous blood was roughly the same as in peripheral venous blood (patients 11–16)

Serum insulin In patients 1–4 the courses of the insulin changes in portal hepatic and peripheral venous blood were roughly parallel (Table III) In patient 1 the insulin level was highest in the portal vein and lowest in the peripheral vein Patients 2–4 however presented a different picture In patient 4 the insulin concentration in the peripheral veins was higher than in the hepatic vein and twice at 67 and 70 min (Table III Fig 1) it even exceeded the insulin concentration in the portal vein In patient 2 the insulin level in the peripheral venous blood was of the same magnitude as or occasionally even higher than in the portal venous blood (Table III) In patient 3 the peak insulin value in the peripheral vein exceeded the peak value in the hepatic vein (Table III)

In patients 5–16 there was again good agreement in general between the time courses of the mean insulin changes in the portal hepatic and peripheral veins The mean portal insulin response in patients 5–10 exceeded the mean portal insulin response in patients 11–16 at one occasion only (2 min after glipizide injection $p<0.05$) The mean insulin peak response in the peripheral venous blood (patie

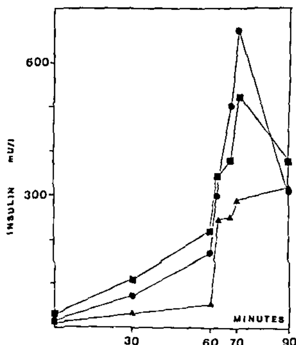


Fig 1 Insulin in portal venous blood (■) hepatic venous blood (▲) and peripheral venous blood (●) in case 4 after peroral glucose and i v tolbutamide (tolbutamide was given 60 min after the glucose intake)

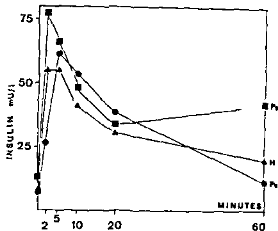


Fig 2 Insulin in portal venous blood (■ Po) hepatic venous blood (▲ H) and peripheral venous blood (● Pe) in case 11 after i v injection of 1 5 mg glipizide

11–16) equalled that in the hepatic venous blood atients 5–10) In patients 11 and 12 the insulin ncentration in the peripheral vein was higher at everal times than the corresponding insulin con centration in the portal vein (Figs 2 and 3)

DISCUSSION

In this study the time courses of insulin changes in peripheral venous blood roughly paralleled the changes in portal and hepatic venous blood This is in accordance with earlier findings (1 2 4 5 8) However it was puzzling that the insulin level in peripheral venous blood sometimes increased above the level in hepatic and portal venous blood when the β cells were stimulated with glipizide or glucose plus tolbutamide

One explanation might be incomplete mixing of blood in the portal vein But if such a mechanism were responsible for the inverse relationship be tween portal and peripheral insulin levels it seems reasonable to expect that on some occasions at least the insulin concentration would be higher in hepatic than in portal venous blood too As this was never the case, incomplete mixing of blood in the portal vein cannot be the sole explanation

We have previously found higher insulin values in peripheral venous blood than in portal blood in pa tients with hepatic cirrhosis and portal caval shunts In these subjects blood glucose in periph eral venous blood sometimes exceeded that in hepatic venous blood (7) In patients 1–4 the blood glucose in peripheral venous blood was always lower than in hepatic venous blood Moreover portography did not show any portal caval shunts

In a recent study we reported that in patients with insuloma a release of previously bound insulin may occur from the liver (3) Other investigations have shown that intra arterial insulin infusion followed by glucose infusions may result in release of previ ously bound insulin from forearm tissue (12) An inverse relationship between portal and peripheral venous insulin levels could probably reflect a re lease of insulin from forearm tissue and thus ex plain the puzzling findings in four patients presented here (nos 2 4 10 and 12) Moreover a release of insulin from peripheral sources would also explain why the mean hepatic venous insulin response to glipizide equalled the mean peripheral venous insu lin response (Table III) The mean peripheral and mean hepatic insulin values admittedly originated from different patients but all patients were given an identical dose of glipizide resulting in roughly equal portal insulin levels (Table III)

The demonstration of a higher insulin concentra tion in peripheral venous blood compared to that in

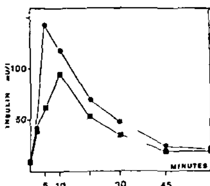


Fig 3 Insulin in portal venous blood (■) and peripheral venous blood (●) in case 12 after i v injection of 1 5 mg glipizide

blood from the hepatic and portal vein in some patients might be explained by acute changes in the binding of insulin to peripheral tissue cells resulting in release of insulin into peripheral venous blood Our data do not allow any analysis of the nature of the change in insulin binding but a decrease in affinity may be suggested

Olefsky and Reaven (11) treated non ketotic diabetics for 1–3 months with sulfonylurea During the treatment hyperglycemia decreased and the number of insulin receptors increased Several studies suggest that insulin itself may be the most important factor in determining its receptor concentration (6 9 10) Possibly chronic sulfonylurea therapy might be accompanied by a frequent release of insulin from peripheral tissues resulting in a decrease in the amount of insulin surrounding or bound to peripheral tissue cells Such a decrease in insulin located in peripheral tissues might in turn lead to an increase in the receptor number and thus explain the result of Olefsky and Reaven

REFERENCES

1 Blachard W G & Nelson N C Portal and peripheral vein immunoreactive insulin concentration before and after glucose infusion Diabetes 19 302 1970
2 — Portal and peripheral vein immunoreactive insulin concentrations following tolbutamide administration Diabetes 20 168 1971
3 Eriksson M Erwald R Hed R Nygren A Patricny J Rojdmark S Sundblad L & Wiechel K L Immunoreactive insulin in portal and hepatic venous blood in patients with insuloma Acta Med Scand 200 145 1976
4 Erwald R Hed R Nygren A Rojdmark S Sundblad L & Wiechel K L Immunoreactive insulin in the portal and peripheral venous blood after intravenous tolbutamide administration Diabetes 20 686 1971
5 — Insulin concentration in portal and peripheral venous blood after oral glucose in human pancreatitis Acta Med Scand 194 103 1973
6 Gavin J R Roth J & Neville D M Jr Insulin dependent regulation of insulin receptor concentrations a direct demonstration in cell culture Proc Natl Acad Sci USA 71 84 1974
7 Hed R Erwald R Nandorf R Nygren A Rojdmark S Sundblad L & Wiechel K L Insulinbestamningar i vena portae vena hepatica och perifer ven vid några kliniska frågeställningar Opusc Med 16 348 1971
8 Horwitz D L Starr I J Mako M E Blanchard W G & Rubenstein A H Proinsulin insulin and C peptid concentrations in human portal and peripheral blood J Clin Invest 55 1278 1975
9 Kahn C R Megyesi K Bar R S Eastman R C & Flier J S Receptors for peptide hormones Ann Intern Med 86 205 1977
10 Olefsky J M The insulin receptor Its role in insulin resistance of obesity and diabetes Diabetes 25 1154 1976
11 Olefsky J M & Reaven G M Effects of sulfonylurea therapy on insulin binding to mononuclear leukocytes of diabetic patients Am J Med 60 89 1976
12 Rasio E Whichelow M J Butterfield W J H & Hichs B H Insulin fixation and glucose uptake by forearm tissue in response to infusions of physiological amounts of insulin in nondiabetic subjects Diabetologia 8 244 1972
13 Soeldner J S & Slone D Critical variables in the radioimmunoassay of serum insulin using a double antibody technic Diabetes 14 771 1965
14 Wiechel K L Blanck C Erwald R Lindberg K & Marions O Vena umbilicalis Nord Med 84 956 1970

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Response to Bicycle Exercise Testing in Long-Standing Juvenile Diabetes

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ABSTRACT Submaximal bicycle ergometry was used in the evaluation of cardiac function in 22 patients with juvenile diabetes and 21 age matched control subjects Six patients had moderate to severe retinopathy and 2 had peripheral neuropathy Half of the patients, but only 3 of the controls were smokers No differences were found in BP serum cholesterol, triglycerides and serum creatinine levels between diabetics and controls None had proteinuria Patients with juvenile diabetes had higher heart rates (HR) at rest as well as during and after exercise than the healthy controls Diabetics also had a reduced HR response to postural changes compared with the controls Five diabetics and one control had a pathological exercise ECG ($0.05<p<0.1$) that may indicate early non symptomatic coronary heart disease The observed changes in HR may be due to autonomic neuropathy

Key words juvenile diabetes mellitus exercise testing autonomic neuropathy non symptomatic coronary heart disease

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The increased risk of coronary heart disease (CHD) in diabetes was pointed out by Levine (14) as early as in 1922 It is now firmly established that diabetics have an increased morbidity and mortality from CHD (12 22) The effect on cardiac function of small vessel disease in myocardial arterioles is not clear Autonomic neuropathy is not uncommon in diabetics and the vagal system is affected more often than the sympathetic nervous system (18)

A graded submaximal exercise test was chosen to study some aspects of cardiac function in young patients with long standing juvenile diabetes and to compare this group with age matched control subjects The primary aim of the study was to diagnose early non symptomatic CHD

SUBJECTS

The 22 patients 6 females and 16 males with juvenile diabetes had a mean age of 21 4 years (S D 3 3) their mean age at onset of diabetes was 5 9 years (S D 3 2) and the mean duration of diabetes 15 4 years (S D 3 5) All patients lived in Oslo and were controlled at regular intervals by one of us Their diabetes was well regulated at the time of study with insulin (Retardin® Leo) twice daily The mean morning dose was 45 2 IU (S D 10 4) and the mean evening dose 19 2 (S D 6 4) Three patients had diminished achilles tendon reflexes 2 also decreased vibration sense indicating peripheral neuropathy Six had retinopathy 5 only mild background retinopathy with microaneurysms and 1 proliferative retinopathy Fourteen of the patients were pupils or students 1 laboratory technician 3 office workers and 4 skilled workers

The controls 6 females and 15 males with a mean age of 20 9 years (S D 2 0) were without known disease Fifteen were students 5 hospital workers and 1 office worker

METHODS

The following laboratory tests were carried out Total serum cholesterol and serum triglycerides in the fasting state protein bound iodine and serum creatinine urine analysis for protein Fasting glucose levels were determined in the control group to exclude manifest diabetes The exercise test was performed as a graded submaximal exercise test on an electrically braked bicycle ergometer (Elema Sweden) Females started on a work load of 300 kpm/min and males on 600 kpm/min The work load was increased by 300 kpm/min every 6th min until a HR of 170–180 beats/min was reached During the test ECG was monitored on a 2-channel oscilloscope Initially a standard 12 lead ECG was recorded in the supine position and thereafter chest head leads (C $H_{1\ 2-4\ 5\ 6}$) (11) were used in the supine and sitting positions during exercise after ½ 1 and 2 min and thereafter every 2nd min After exercise ECG was recorded in the sitting position after ½ min and in the supine position after 1 2 4 and 10 min Finally a standard 12 lead ECG was recorded

The ECGs were evaluated using the criteria of the modified Minnesota Code (1) This Scandinavian modification of the code includes ST depression of a slowly ascending type (S type) in which the T wave starts below the isoelectric line To be considered significant ST depressions should be present in more than one registration and not disappear at increasing work loads

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Abbreviations CHD=coronary heart disease HR=heart rate

Table I *Some clinical and laboratory data on 22 patients with juvenile diabetes and 21 control subjects*
Mean values S D in parentheses

	Controls	Diabetics	*p*
No of females	6	6	
No of males	15	16	
Age (y)	20 9 (2 0)	21 4 (3 3)	
Height (cm)	177 0 (9 3)	174 5 (9 0)	
Weight (kg)	69 3 (11 9)	66 2 (7 5)	
No of cigarette smokers	3	11	<0 001
No of cigarettes/day	10 3	9 7	
Systolic BP (mmHg)	123 1 (12 3)	123 0 (8 1)	
Diastolic BP (mmHg)	80 0 (7 7)	76 1 (7 4)	
Cholesterol (mg/100 ml)	210 8 (30 2)	203 0 (49 1)	
Triglycerides (mg/100 ml)	52 6 (14 3)	75 5 (105 0)	
Protein bound iodine (μg/100 ml)	6 2 (1 1)	5 9 (1 0)	
S-creatinine (mg/100 ml)	0 90 (0 2)	0 93 (0 13)	
Proteinuria	0	0	
Fasting glucose (mg/100 ml)	83 8 (8 9)		
Pathologic exercise ECG	1	5	0 05<*p*<0 10

Statistical methods

Mean and S D were calculated according to standard formulas Student s *t* test was used to evaluate the significance of differences between patients with juvenile diabetes and control subjects

RESULTS

Clinical and laboratory data are given in Table I e number of cigarette smokers was significantly igher among diabetics than controls Otherwise no significant differences were found

Exercise test The patients with juvenile diabetes had significantly higher HR than the controls both at rest and during and after exercise as shown in Table II and Fig 1 The mean change in HR when changing from the supine to the sitting position was smaller in the diabetic (3 3 beats/min) than in the control group (9 2 beats/min) the difference being statistically significant ($p<0$ 05) The mean total work performance was much higher in controls than patients (Fig 2) as was their mean work level at a HR of 170 (Table III) None of the subjects experienced serious symptoms during exercise The test was discontinued in all subjects because the desired HR had been reached

Five of the diabetic patients and one control subject had a positive exercise ECG during work and none after exercise The ECG changes were of the S type in one diabetic also of the horizontal or

Table II *Mean HR (beats/min) at rest and during exercise in 22 diabetics and 21 normal controls*
S D in parentheses

	Controls	Diabetics	*p*
Rest			
Supine	71 5 (10 3)	87 3 (13 1)	<0 0005
Sitting	60 7 (13 9)	91 0 (11 3)	<0 01
Exercise (kpm/min)			
300	126 2 (19 7)	133 8 (9 6)	NS
600	133 4 (24 0)	147 1 (15 5)	<0 05
900	153.5 (18 4)	170 1 (11 8)	<0 005
1200	167 2 (11 9)	176 0 (15 8)	<0 025
After exercise (min)			
Sitting ½	150 1 (18 4)	157 8 (8 5)	<0 05
Supine 1	117 2 (15 1)	128 4 (11 4)	<0 01
Supine 2	100.3 (14 7)	108 7 (8 8)	<0 01
Supine 4	92 6 (9 2)	102 0 (7 6)	<0 0025
Supine 10	85.3 (9 1)	93 5 (7 2)	<0 0025

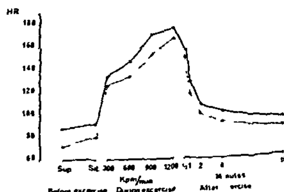


Fig 1 Mean HR in diabetics (—) and controls (---) before during and after exercise *Sup* =supine *Sit* =sitting position

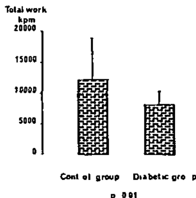


Fig 2 Mean total work in diabetics and control subjects

downward sloping type (I type) with an ST segment depression of 1–3 mm The changes occurred at work loads of 300–1200 kpm/min and HRs of 125–180 One further diabetic—not with positive ECG—had S type ST depressions which disappeared at higher work levels Thus 23% (5/22) of the diabetics had a positive exercise ECG against 5% (1/21) of the controls The difference is not statistically significant (0 05<p<0 10)

DISCUSSION

The association between diabetes and CHD is well known In recent years attention has also focused on the autonomic neuropathy and abnormal cardiac reflexes which are found in a great number of diabetic patients (16 21 23)

Vagal neuropathy is most dominant and can be demonstrated by tests which stimulate or inhibit vagal function The response to the Valsalva manouvre or the cardiac beat to-beat variation can be used as objective measures of vagal function Numerous studies report a decrease in vagal function in a great number of diabetic patients (2 4 10 23) Tachycardia can therefore be attributed to vagal neuropathy (6 7 8 10 23) In the present study patients with juvenile diabetes had significantly higher HR both at rest and during and after submaximal exercise (Table II Fig 1) This higher HR in our patients may reflect an autonomic *neuropathy and possibly poorer physical fitness* The smaller rise in HR in the diabetic group when changing from the supine to the sitting position is however consistent with impairment of baroreceptor reflexes (4) and cannot be explained by a lower work capacity

Exercise tolerance was lower in juvenile diabetics than in control subjects (Table III Fig 2) but interpretation is rendered difficult by the fact that the excercise was stopped when a HR of 170–180 had been reached The vagal neuropathy can partly explain that the diabetics reached a HR of 170–180 at a lower work level than controls The lower tolerance may therefore not be solely due to a reduced physical working capacity

The Scandinavian modification of the Minnesota Code was used to diagnose positive exercise ECGs Prospective studies (20) have shown that ST depressions of the S type have a clear prognostic value for the development of manifest CHD Eriksen et al (9) have correlated ECG changes during and after exercise to coronary angiography in presumably healthy men They found that ECG changes of the S type during exercise had the same diagnostic significance as ST T changes of the I type In postexercise recordings however I type changes had a higher diagnostic performance The coronary artery changes demonstrated by angiography were of the same frequency in asymptomatic subjects with S and I type changes during exercise Of our diabetic patients 4 had S type and one I type changes during exercise with no changes after exercise

Persson (19) has recently published a follow up on diabetic and control subjects who had undergone a bicycle test 9 years earlier (13) He found more infarcts and higher morbidity in the diabetic than in the control group More precisely of the 12 subjects who had suffered a myocardial infarction 9 had had pathological work ECGs 9 years previously compared to only 27% of the total diabetic population Thus pathological exercise ECG is an ominous prognostic sign in diabetics Bellet and Roman (3) found an incidence of 22 3% positive exercise ECGs in 85 diabetics aged 20 or more (the majority

Table III *Total work performed (kpm) and work level (kpm/min) at a HR of 170 beats/min in 22 diabetics and 21 controls*

	Controls	Diabetics	p
Mean total work	12 380	8 281	<0 01
S D	6 889	2 258	
Mean work level at HR 170	1 142	954	<0 01
S D	278	176	

over 40 years old) compared to 8–12% in non diabetic subjects Younger diabetics (<40 years) had almost the same incidence (17 4%) as older diabetics (24 2%) Similarly Levitas and Kristal (15) found 20% positive exercise ECGs in diabetics aged 20–40 The present study confirms the higher frequency of pathological work ECGs in diabetics although our patients were 10 years younger They also had had diabetes for a longer time

Smoking common in juvenile diabetics as this study confirms is a serious risk factor for CHD There is also evidence that smoking adversely influences the development of diabetic retinopathy (17) Smoking should accordingly be discouraged in diabetics

Diabetics have higher mortality and morbidity from CHD than non-diabetics (2) Exercise ECG can diagnose CHD in diabetics and in normals before it becomes symptomatic and may be a valuable tool for early diagnosis and perhaps therapy Few papers however have been published previously on exercise ECG in diabetics (3 5 9 13 15) and more studies with follow up are needed

REFERENCES

1 Åstrand I Areskog N H Carlsten A Grewin K E Kaijser L Malmstrom G Punsar S Blomquist G Bjerkelund C Furberg C Hansen F Kallio V Nordgren L Pyorala K & Thulesius O The Minnesota Code for ECG classification Adaptation to CR leads and modification of the code for ECGs recorded during and after exercise Acta Med Scand (Suppl) 481 1967

2 Baldwa V S & Ewing D J Heart rate response to Valsalva manoeuvre Reproducibility in normals and relation to variation in resting heart rate in diabetics Br Heart J 39 641 1977

3 Bellet S & Roman L The exercise test in diabetic patients as studied by radioelectrocardiography Circulation 36 245 1967

4 Bennett T Hosking D J & Hampton J R Cardiovascular control in diabetes mellitus Br Heart J 2 585 1975

5 Campbell I W McGarry S Smith D N Neilson J M & Clarke B F Continuous electrocardiographic recordings during exercise in young male diabetics Br Heart J 37 277 1975

6 Christensen N J Plasma catecholamines in long term diabetics with and without neuropathy and in hypophysectomized subjects J Clin Invest 51 779 1972

7 Christensen N J & Gundersen H J G Plasma volume and adrenergic activity after intravenous insulin In Current topics in diabetes research (ed J S Bajaj) p 112 9th Congress of International Diabetes Federation Amsterdam Excerpta Medica 1976

8 Eichorst H Beitrage zur Pathologie der Nerven und Muskeln Arch Path Anat 127 1892

9 Erikssen J Enge I Forfang K & Storstein O False positive diagnostic tests and coronary angiographic findings in 105 presumably healthy males Circulation 54 371 1976

10 Ewing D J Campbell I W Burt A A & Clarke B F Vascular reflexes in diabetic autonomic neuropathy Lancet 2 1354 1973

11 Holmgren A & Strandell T On the use of chest head leads for recording of electrocardiogram during exercise Acta Med Scand 169 57 1961

12 Jarrett J Diabetes and the heart Coronary heart disease Clin Endocrinol Metabol 6 389 1977

13 Karlefors T Circulatory studies during exercise with particular reference to diabetics Acta Med Scand (Suppl) 449 1966

14 Levine S A Angina pectoris Some clinical considerations JAMA 79 928 1922

15 Levitas I M & Kristal J J Stress exercise testing of the young diabetic for the detection of unknown coronary artery disease Isr J Med Sci 8 845 1972

16 Lloyd Mostyn R H & Watkins P J Defective innervation of the heart in diabetic autonomic neuropathy Br Med J 2 15 1975

17 Paetkau M E Boyd T A S Winship B & Grace M Cigarette smoking and diabetic retinopathy Diabetes 26 46 1977

18 Page M McB & Watkins P J The heart in diabetes Autonomic neuropathy and cardiomyopathy Clin Endocrinol Metabol 6 377 1977

19 Persson G Cardiovascular complications in diabetics and subjects with reduced glucose tolerance Acta Med Scand (Suppl) 605 1977

20 Punsar S Pyorala K & Siltanen P Classification of electrocardiographic ST segment changes in epidemiological studies of coronary heart disease Ann Med Fenn 57 53 1968

21 Sharpey Schafer E P & Taylor P J Absent circulatory reflexes in diabetic neuritis Lancet 1 559 1960

22 Westlund K Mortality of diabetics Universitetsforlaget Oslo 1969

23 Wheeler T & Watkins P J Cardiac denervation in diabetes Br Med J 4 584 1973

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A Stroke Unit in a Medical Department

Organization and the First 100 Patients

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ABSTRACT A non intensive stroke unit of 10 beds has been started in the Medical Department Serafimerlasarettet, Stockholm The aim is to make diagnostic and therapeutic studies in unselected stroke patients Patients with suspect cerebrovascular disease in the Casualty Department are admitted to the unit non selectively and without any age limit Relevant physical findings and laboratory data are followed and registered by code on special charts to make evaluation by computer possible A preplanned investigative programme is adhered to Strict criteria for diagnosis and treatment are followed The experience and results from the first 100 patients treated in the Stroke Unit indicate that the unit is a good basis for both education and research

Key word stroke unit

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Stroke is one of the major causes of death in industrialized countries and the main cause of permanent disability In Sweden as in several other countries most patients with stroke are treated in medical departments and only around 10% usually patients below 60 years of age are cared for in departments of neurology

To study the value of different diagnostic investigations and therapeutic interventions stroke intensive care units or neurovascular care units have been established during the last ten years mainly in the US The aim of the work at our non intensive Stroke Unit has been to form a basis for studies regarding diagnostic and therapeutic procedures and to create an investigative programme for non selected patients of all ages with acute cerebrovascular disease (CVD) The present report contains a description of the routines in the unit and the results from the first 100 patients treated there

PATIENTS AND METHODS

One hundred consecutive patients with CVD treated in the Stroke Unit at the Medical Department Serafimerlasarettet were included in the present study Serafimerlasarettet serves a population of 120000 inhabitants in greater Stockholm and around 300 patients with stroke are admitted every year The Stroke Unit has been calculated to care for up to 2/3 of these patients

Criteria for admission

1 *Transitory ischaemic attacks* (TIA) Patients with one or more episodes of focal neurological deficit with a duration of less than 24 hours within the last month Attacks of vertigo or syncope without focal neurological deficit are not included 2 *Progressive and manifest stroke* Patients with acute onset of focal neurological deficit during the last week and without preceding trauma to the head

The Stroke Unit

In Oct 1976 a five bed unit for women with CVD was arranged as part of an ordinary medical ward and in April 1977 five beds for men were added Patients fulfilling the admission criteria in the Casualty Department are admitted to the Stroke Unit in a non selective manner The patients are examined physically in the Casualty Department on admission to the Stroke Unit on the fourth day after admission and before discharge New examinations are made if signs of deterioration appear All patients stay in the unit until discharge The resources for general care in the unit do not differ from those in other medical wards no facilities for intensive care are available

Examinations

Lumbar puncture is performed routinely preferably around 24 hours after the onset of symptoms Spectrophotometric analysis is made according to the procedure described by Kjellin and Soderstrom (8) by which bleeding patterns may be recognized In patients with an absorption of >0 030 at 410 nm a new lumbar puncture is performed after one week to exclude or verify a bleeding pattern

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Abbreviations CVD=cerebrovascular disease TIA=transitory ischaemic attacks CSF=cerebrospinal fluid

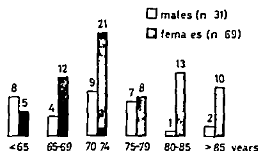


Fig. 1. Age and sex distribution of 100 consecutive stroke patients.

X-ray of the skull and echoencephalography (10) are performed routinely as soon as possible after admission. Brain scans (2) are performed if possible 3–6 and 10–14 days after the onset of symptoms. Computerized axial tomography was not available during the present study.

Arch angiography is performed in 1) All patients with an ischaemic stroke <55 years of age and without any general contraindications (myocardial infarction during the last three months, unstable angina, suspected brain stem infarction, allergic reactions to contrast, a very low prothrombin value or total hemiparesis and aphasia or disturbed consciousness). 2) Patients 55–75 years old with (*a*) TIA from the carotid territory, (*b*) manifest stroke with minor neurological deficit, i.e. independence in daily activities, (*c*) atrial fibrillation and a history of prior cerebral embolism affecting the same vascular region but without a history of peripheral embolism.

Carotid arteriography is performed in (*a*) patients <55 years of age when arch angiography has shown no significant changes in the relevant artery, (*b*) in all patients in whom subdural haematoma, subarachnoid haemorrhage or cerebral tumour is suspected.

Diagnostic criteria

1. *Cerebral haemorrhage* (ICD 431). Macroscopically haemorrhagic cerebrospinal fluid (CSF) or bleeding pattern at spectrophotometry. 2. *Atherothrombotic brain infarction* (ICD 432, 433). Patients with findings in accordance with an ischaemic lesion at spectrophotometry. 3. *Cerebral embolus* (ICD 434). Sudden onset of symptoms in patients with atrial fibrillation, valvular disease or significant atherosclerotic changes in the relevant carotid artery. Findings at spectrophotometry in accordance with an ischaemic lesion. 4. *TIA* (ICD 435). Focal neurological deficit with a duration of less than 24 hours. 5. *Unspecified acute CVD* (ICD 436). Patients with CVD not fulfilling the above criteria.

Anticoagulant and antithrombotic treatment

TIA, carotid territory. (*a*) Two or more TIA during the last 48 hours and CSF without xantochromia at spectrophotometry. Patients <75 years of age and without contraindications receive heparin and warfarin. Patients ≥75 or <75 years with contraindications to anticoagulant treatment receive acetylsalicylic acid (Premaspin) 1 g twice daily. (*b*) One or more TIA during the last month. Patients <75 years of age and without contraindications receive warfarin. Patients ≥75 or <75 years with contraindications to anticoagulants receive acetylsalicylic acid as above. Surgery is suggested to patients ≤75 years of age with TIA from the carotid territory and who show a ≥50% stenosis or ulcerated plaques of the relevant artery. *TIA, vertebral territory.* Acetylsalicylic acid as above.

Progressive stroke. Patients <75 years of age with progressing focal neurological deficit within the last 24 hours and without total hemiparesis and aphasia, sopor or coma and without xantochromic CSF receive heparin and warfarin. Patients who are older or have contraindications to anticoagulant treatment receive acetylsalicylic acid.

Manifest ischaemic stroke (ICD 432, 433, 434). These patients receive acetylsalicylic acid as mentioned above. If the patient shows atrial fibrillation and is ≤75 years warfarin is given.

Cerebral haemorrhage. Symptomatic treatment. For patients with a subarachnoid haemorrhage contact is established with a neurosurgical clinic.

Treatment of high blood pressure

BP is measured regularly during the first three days and then intermittently. No treatment is given initially but patients on previous antihypertensive treatment maintain their medication. If two consecutive BP measurements during the third day exceed the following limits, treatment is usually started with thiazides and/or β-receptor blocking agents: patients >65 years 180/105 mmHg, 40–65 years 170/100 mmHg and <40 years 160/90 mmHg.

Previous diseases

Stroke—a report of CVD preferably treated in hospital. Myocardial infarction—a report of a myocardial infarction usually verified from a hospital record. Angina pectoris—substernal pain with or without radiation or left sided precordial chest pain radiating into the left arm. The pain should be related to exertion and disappear within 10 min of rest or taking nitroglycerine. Hypertension—a history of high BP treated with antihypertensive agents or a verified history of high BP without treatment. Heart failure—typical symptoms or a history of digitalis therapy or diuretic therapy not given for hypertension.

Function groups as regards the ability to move. F1=totally independent. F2=the patient walks with technical aid. F3=the patient walks only with live support. F4=bedridden.

Table I. *Previous diseases and smoking habits*

	%
CVD (stroke, TIA)	24
Hypertension	49
Heart failure	40
Atrial fibrillation	18
Angina pectoris	17
Myocardial infarction	8
Intermittent claudication	5
Diabetes	10
Hyperlipdaemia	5
Smokers	21

Table II *Diagnoses and mortality*

	No of pats	Mortality (%)
Cerebral haemorrhage	12	50
Atherothrombotic brain infarction	54	15
Cerebral emboli	24	21
TIA	7	–
Unspecified CVD	3	–
Total	100	19

RESULTS

The 100 patients comprised 69 women and 31 men Their mean age was 74 years (women 75 men 71 range 51–92) The age and sex distributions are shown in Fig 1 Mean delay between onset of symptoms and admission to hospital was 20 hours (range 1/2 h–1 week) Seventy six per cent of the patients arrived within 24 hours and only 7% after more than 48 hours The average duration of the stay in the Stroke Unit was 21 days (range 1–59)

Significant previous diseases and smoking habits are shown in Table I Half of the patients had a history of high BP and one fourth had had CVD Eighteen per cent of the patients had chronic atrial fibrillation during the hospital stay atrial fibrillation was registered in another 12% The mean age of all patients with atrial fibrillation was very high 77 years

Distributions of diagnoses and mortality are shown in Table II Atherothrombotic brain infarctions predominated (54%) whereas few intracerebral haemorrhages were diagnosed Total hospital mortality was 19% with the highest mortality among patients with intracerebral haemorrhages

Investigations

The frequency with which planned investigations were carried out and the proportion of positive findings are shown in Table III Six patients died before lumbar puncture one patient refused and in one it could not be performed due to technical difficulties Analysis of CSF including spectrophotometry showed an absorption of >0 030 at 410 nm in 17 out of 92 patients but a bleeding pattern or autopsy findings of an intracerebral haemorrhage were verified in 12 patients only In the remainder the spectrophotometric pattern was in accordance with an ischaemic lesion

No fractures were diagnosed on the plain skull X rays Four patients had a dislocation of the midline structures exceeding 3 mm all these patients had intracerebral haemorrhages

The first brain scans were usually performed a few days after admission Of the 19 patients who were not scanned 14 died shortly after admission The scans showed pathologic changes in 42 out of 81 patients The scans were pathologic in all six patients with cerebral haemorrhage whereas 32 out of 65 patients (49%) with cerebral infarction had positive scans Two out of seven patients with TIA showed a pathologic uptake

Using the above indications for angiography an arch angiogram was performed in nine and a carotid arteriography in three patients Only one of the patients showed an ulcerated plaque in the relevant artery and subsequently underwent thrombendarterectomy Another patient with a 50% stenosis developed a manifest stroke before the scheduled operation

Anticoagulant treatment

In 24 patients the diagnosis was cerebral embolus but warfarin treatment was instituted in only six (25%) of them The main reason for this discrepancy was the high mean age of the patients with atrial fibrillation Seven patients had a diagnosis of TIA and four of them were given warfarin, one patient was given heparin initially because of repeated TIA during the last 48 hours Only one patient was given heparin because of a progressive stroke

Function groups

On admission the majority of patients were either bedridden (F4) or could walk only with live support

Table III *Frequency of diagnostic investigations and positive findings*

	No of investigations	Positive findings (%)
Lumbar puncture	92	18
X ray of the skull and echoencephalography	94	4
Brain scanning	81	52

Table IV *Function groups on admission and at discharge*

On admission		At discharge F 1–2	F 3	F 4	Deceased
F 1–2	34	31	2	1	–
F 3	24	18	3	–	3
F 4	42	9	8	9	16
Total	100	58	13	10	19

(F 3) At discharge most patients could walk independently or with a technical aid (F 1–2) Patients with haemorrhages had the worst outcome as regards both function and mortality There was a definite association between function group on admission and prognosis (Table IV) None of the patients in F 1–2 on admission died during the hospital period

Time and mode of death

Nineteen patients died during the hospital period The mean duration of their stay in the Stroke Unit was nine days (range 1–27) Autopsy was performed all but one of the deceased Cerebral infarction diagnosed in 12 and intracerebral haemorrhage six patients Five of the latter had haemorrhages extending into the ventricles and four of them died within 24 hours after admission The patients with ischaemic lesions died after a mean period of 10 days A recent myocardial infarct was found in eight (44%) of the deceased patients considered a terminal event in four

DISCUSSION

There has been a reluctant attitude towards the establishment of stroke units in Sweden One reason has certainly been the lack of evidence that acute stroke mortality is significantly reduced in such units even when they have equipment for intensive care (4 5 6 7 13 14) Still stroke patients do constitute a very important group in our medical wards and during the last few years the need for stricter investigative programmes and more active treatment of these patients has been stressed repeatedly (1 3 11 16)

The majority of the first 100 patients admitted to our non intensive Stroke Unit arrived within 24 hours of the onset of symptoms Their mean age 74 years was high compared with that in several other studies the absence of an age limit and the dominance of women due to an earlier start for the female part of the unit account for this finding

Previous cardiovascular disease was very common among our patients As in other studies only about 1/4 of all patients had no history of cardiac disease (13) Definite comparisons are difficult to make because of differences in age distribution and diagnoses in the study groups The number of patients with atrial fibrillation was remarkably high 30% compared to that reported by other authors usually a figure around 12% is mentioned (13) Considering the high prevalence of cardiac disease it is hardly surprising that cardiac complications were often registered in the present patient group The poor prognosis of stroke patients with concurrent heart disease is well known (5) and our findings again stress the importance of careful observation of such complications Few patients developed an acute myocardial infarction simultaneously with the stroke but both old and recent myocardial infarcts were common findings at autopsy

The investigative programme was carried through in almost all patients Spectrophotometry of the CSF was the main diagnostic tool for differentiating between haemorrhagic and ischaemic lesions (8) Skull X ray and echoencephalography were normal in 9 out of 10 patients and no skull fractures were diagnosed The results of these investigations did not influence diagnosis or treatment in any patient Comparatively few of our patients showed a midline shift (17) This may partly be du to the fact that echoencephalography was not performed in some of the patients with intracerebral haemorrhage who died shortly after admission and to the circumstance that the investigation was rarely made more than once in each patient As expected the brain scans were more often positive in patients with cerebral haemorrhage than in those with cerebral infarction (2) They did not however add significantly to the diagnostic decisions in the present patient group In no patient was a cerebral tumour discovered

Angiography was performed in 12% of the patients Only one patient was subsequently operated on The low frequency of arteriographies may be ascribed to the advanced mean age of the patients

and to the fact that such investigations were proposed only when operative intervention was considered possible The high mean age of the patients also explains the low number of patients on anticoagulant treatment Only 10 out of 31 patients with a diagnosis of cerebral embolus or TIA received such medication

The distribution of diagnoses 78% of the patients having a cerebral infarction is comparable to that in other studies (5 13) None of our 100 patients had subarachnoid haemorrhage this was due to the fact that patients with suspected subarachnoid haemorrhage were transferred directly after lumbar puncture from the Casualty Department to a neurosurgical clinic As expected a poor short term prognosis was noted in patients with cerebral haemorrhage mortality rate 50% The total mortality rate of 19% is comparable to that in other similar studies (5 6 12 13) and so are the proportions of survivors who walked independently (72%) or were bedridden (12%) at discharge (5 12 15) In spite of advanced age most of the survivors improved markedly during their hospital stay

The experience accumulated by the medical and nursing personnel during the first year of the Stroke Unit constitutes a valuable resource for future work Our aim for the running in period was to make routines work It is our opinion that a stroke unit of the present kind creates a good basis for education and research and promotes interest in and engagement for the care of stroke patients

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REFERENCES

1 Biorck G Britton M de Faire U & Wester P O Ischemiska cerebrovaskulara lasioner Etiologi och riskfaktorer Lakartidningen 73 3971 1976

2 Boller F Patten D H & Hower D Correlation of brainscan results with neuropathological findings Lancet 1 1143 1973

3 Boren A Behandlingen på centrallasarett av cerebrovaskulara sjukdomar Lakartidningen 72 234 1975

4 Cooper S W Livet I A & Woolsey F M Jr Establishment and operation of a combined intensive care unit for patients with cardiac and cerebrovascular disease N Y State J Med 72 2215 1972

5 Drake W E Jr Hamilton M J Carlsson M & Blumenkrantz J Acute stroke management and patient outcome The value of neurovascular care units (NCU) Stroke 4 933 1974

6 Haerer A F Smith R R & Currier R D The Mississippi regional medical program stroke unit Critique and follow up of the first 250 patients admitted South Med J 64 951 1971

7 Kennedy F B Pozen T J Gabelman E H Tuthill J E & Zaentz S D Stroke intensive care—An appraisal Am Heart J 80 188 1970

8 Kjellin K G & Soderstrom C E Diagnostic significance of CSF spectrophotometry in cerebrovascular disease J Neurol Sci 23 359 1974

9 Lavy S Yaar I Melamed E & Stern S The effects of acute stroke on cardiac functions as observed in an intensive stroke care unit Stroke 5 775 1974

10 Leksell L Detection of intracranial complications following head injury Acta Chir Scand 110 301 1955

11 Link H Avsevart förbattrat omhandertagande av patienter med cerebral insult efterlyses Lakartidningen 71 4443 1974

12 Marqvardsen J The natural history of acute cerebrovascular disease Munksgaard Copenhagen 1969

13 Norris J W & Hachinski V C Intensive care management of stroke patients Stroke 7 573 1976

14 Pitner S E & Mance C J An evaluation of stroke intensive care Results in a municipal hospital Stroke 4 737 1973

15 Rankin J Cerebral vascular accidents in patients over the age of 60 Part II Prognosis Scott Med J 2 280 1957

16 Silfverskiold B Slaganfall—Har vi råd avstå från specialiserad vård? Läkartidningen 73 3108 1976

17 deVlieger M & Krull G H Echo-encephalography in the diagnosis of cerebral vascular diseases In Cerebral vascular disease Grune and Stratton New York 1968

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Haemolytic Anaemia in Alcohol Abuse

A Review of 14 Cases

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ABSTRACT Clinical and haematological data on 14 patients (8 women and 6 men) with alcohol induced haemolytic anaemia and mild to moderate liver injury are presented Nine of the patients were obvious drinkers, while 5 were socially well adjusted individuals in whom alcohol dependence was not suspected on admission to hospital Four patients presented with a typical Zieve s syndrome Two further patients had a moderate transient stomatocytosis as assessed by microscopy of dried blood smears and by scanning electron microscopy The majority of the patients however, did not fit into any of the syndromes proposed in the literature Indeed, the validity of both Zieve s syndrome and the ' transient stomatocytosis with hemolysis syndrome is questioned

Key words haemolysis alcohol Zieve s syndrome stomatocytosis

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Anaemia is often found in alcoholic subjects after prolonged excessive ingestion of ethanol Alcohol may depress erythrocyte production (23) and vacuolated red cell precursors (19) or sideroblasts (11) may be found in the bone marrow Megaloblastic changes may be observed in connection with malnutrition and folate deficiency (8) but have also been noted in patients with normal folate levels (16) Furthermore blood loss due to gastrointestinal bleeding is common in these individuals Finally alcohol has been implicated in the development of haemolytic anaemia

In 1955 Jandl (13) described haemolysis thought to be due to increased splenic red cell destruction in 20 cirrhotic subjects Increased red cell sequestration in the spleen has also been found to occur in the spur cell anaemia characteristically seen in advanced alcoholic liver disease (22) A different kind of abnormal erythrocyte morphology was later described by Douglass and Twomey (7) who found transitory stomatocytosis with haemolysis in four male alcoholics with moderate liver disease and normal spleen size In 1958 Zieve (26) described the syndrome that came to bear his name comprising haemolytic anaemia hyperlipidaemia and jaundice in individuals with moderate alcoholic liver disease and no evidence of splenic hyperactivity Subsequently a number of reports confirming the existence of this syndrome in its complete (2 9 10) or partial (17) form have appeared in the literature although others (4) have been reluctant to recognize its existence Extracorpuscular (2) as well as intracorpuscular (9) mechanisms have been proposed to explain the haemolysis seen in this postulated syndrome Eichner and Hillman (8) in a carefully designed study demonstrated that differences in the timing of the medical investigation in relation to alcohol ingestion and abstinence may reveal different haematologic patterns in a given patient

In the present report data on 14 patients with ethanol induced haemolytic anaemia are presented and discussed in relation to the classification proposed in the literature

PATIENTS AND METHODS

The patients 8 women and 6 men aged 36–60 years (Table I) were admitted to a general medical ward under a variety of diagnoses Chronic alcohol abuse was evident on admission in 9 of the patients 5 were socially well adapted Alcohol dependency was not suspected initially in any of the latter 5 individuals and was revealed only on repeated questioning of the patients or their relatives All the patients had biochemical and histological evidence of mild to moderate liver disease Twelve patients had steatosis and 6 of them had in addition slight to moderate periportal fibrosis (Department of Pathology Ullevål Hospital) Two patients (nos 4 and 14) fulfilled the pathological criteria for an incipient cirrhosis Four patients had an acute alcoholic hepatitis superimposed on pathological changes consisting of steatosis fibrosis or cirrhosis Spleen size was evaluated by abdominal palpa

Table I *Characteristics of patients with alcohol induced haemolytic anaemia*

$t_{\frac{1}{2}}$Cr=Half life for the disappearance of ^{51}Cr from the circulation Z=Zieve s syndrome pZ=partial Zieve s syndrome S=stomatocytosis n i =not investigated

Pat no	Sex	Age (y)	Alco-holism evident	Hb (g/100 ml)	Reticulocytes %	Reticulocytes On day no	Erythro-poiesis (%)	$t_{\frac{1}{2}}$Cr (d)	Total bilirubin (μmol/l)	Hyper lipid aemia	Syn drome
1	♂	57	+	11 3	3 3	10	52	9½	305	+	Z
2	♀	53	–	9 5	3 0	7	46	13½	10	+	pZ
3	♀	57	–	10 4	2 5	1	43	15	13	+	pZ
4	♀	43	+	11 9	4 5	6	45	19	23	–	–
5	♀	46	+	9 9	5 2	15	51	21	17	+	pZ S
6	♀	58	+	9 8	2 3	11	49	19	25	–	–
7	♂	45	–	9 6	5 5	15	49	n i	48	+	Z
8	♀	46	+	7 2	12 2	5	50	12½	75	–	pZ
9	♂	46	–	15 3	2 1	1	39	15	55	–	pZ
10	♂	40	–	11 9	22 0	1	41	19	13	–	–
11	♀	36	+	8 2	4 7	6	54	19½	112	+	Z
12	♂	60	+	8 7	2 7	22	8	14½	4	–	S
13	♀	42	+	10 7	3 3	6	38	15	75	+	Z
14	♂	57	+	8 8	2 8	3	40	18	48	–	pZ
Normal laboratory values				♂ 12 5–16 5 ♀ 11 5–15 5	0 5–2 0		15–30	27–30	3–25		

tion and by ^{99m}technetium scanning (Isotope Laboratory Ullevål Hospital) The spleen was not palpable in any of the patients although slight scintigraphic enlargement was noted in patient 6 All had been drinking until the time of admission but were abstinent during the study period

Blood samples were analyzed by routine methods at the artment of Clinical Chemistry Ullevål Hospital

rythropoiesis was evaluated by microscopy of May runwald-Giemsa stained bone marrow aspirates count ing at least 500 nucleated cells The normal percentage of erythrocyte precursors is taken to be 15–30

Erythrocyte survival was determined by the radioactive chromium technique (Isotope Laboratory Ullevål Hospi tal) The results are expressed in terms of half life for the disappearance of ^{51}Cr from the circulation ($t_{\frac{1}{2}}$Cr) normal range 27–30 days External body surface counting was performed in order to detect increased red cell destruction in the spleen

Erythrocyte morphology was assessed by microscopy of May-Grunwald-Giemsa stained dried smears of peripheral blood anticoagulated with EDTA Red cell morphology was judged in that part of the smear where the cells are *thought to be best preserved i e approximately 1 cm* from its tail

For *scanning electron microscopy* (carried out at the Electron Microscopy Unit for Biological Sciences Uni versity of Oslo) freshly drawn heparinized blood was fixed (1 4) in a 1% glutaraldehyde solution buffered by 0 1 M sucrose/cacodylate (pH 7 40) Small samples were transferred to coverglass coated with polylysine (18) and the cells were allowed to sediment The speci mens were dehydrated with graded ethanols increasing to 100% dried by the critical point method using CO_2 (1) and coated with 400 Å gold in a Polaron E 5000 diode sputtering system We found no evidence of morpho logical artefacts due to the fixation and dehydration pro cedures (3) as can be seen from the morphology of red cells in samples from control (normal) blood (Fig 3a) The cells were examined in a Jeol JSM 35 scanning elec tron microscope at 10 kV

RESULTS

Evidence for the presence of haemolysis induced by alcohol

The Hb values on admission are shown in Table I All but 2 patients had slight to moderate anaemia Bone marrow aspiration was performed within 2 days and erythrocyte survival studies were then initiated No marked changes in the Hb level were recorded during the red cell survival study period The reticulocyte percentages given in Table I rep resent the highest count observed in each patient It is evident that several days elapsed (median 6 range 1–22) before the peak reticulocytosis was reached Mean corpuscular volume was increased on admission in all but one patient (median 106 range 91–133)

In most patients haemolysis was suspected on the basis of reticulocytosis and an increase of eryth rocyte precursors in the bone marrow and was confirmed by red cell survival studies In patient 7 the diagnosis was based on a marked fall in the Hb

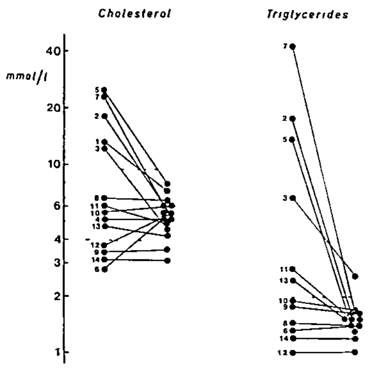


Fig 1 Fasting serum cholesterol and triglyceride levels on admission and after 2–4 weeks Cholesterol only was measured in patients 1 and 4 The numerals to the left of the symbols indicate patient number

level despite evidence of an increased erythropoiesis and in the absence of blood loss As can be seen from Table I $t_{1/2}$Cr was moderately to severely shortened The percentage of erythrocyte precursors in the marrow was increased above normal in all but one patient (no 12) whose anaemia probably resulted from a moderate haemolysis in the presence of marked suppression of erythropoiesis As assessed by external radioactive counting erythrocyte breakdown in the spleen was not increased in any of the patients

Consistently negative Coombs test cold agglutinin test and sucrose lysis test provided evidence against other causes of the haemolysis Tests for occult blood in the stools were also negative The signs of haemolysis subsided during 2–4 weeks of abstinence in all and recurred in 2 patients who resumed their drinking habits

Serum lipids

Fasting serum cholesterol and triglyceride values on admission and after 2–4 weeks are shown in Fig 1 Four patients had increased levels of both lipid fractions while one had an increase in cholesterol only and another in triglycerides only

Erythrocyte morphology

Several patients manifested abnormal red cell morphology commonly found in liver disease i e macrocytes target cells and occasional spherocytes Significant numbers of spur cells were not observed In two patients there was a moderate increase (3 and 4% respectively) of stomatocytes above the 0 5% level or less found in normal individuals Stomatocytes are red cells in which the usual central pallor is replaced by a mouth like slit or stoma when flattened on a slide (Fig 2) while the bowl shaped configuration clearly emerges on electron microscopy scanning (Fig 3*b* and *c*) Although the stomatocyte enumeration on dry blood smears is somewhat arbitrary the results correlated well with those obtained by scanning electron microscopy

Classification of the patients

An attempt was made to classify the patients according to the various syndromes described in the literature (Tab I) Four patients manifested a fully developed Zieve s syndrome 6 partial Zieve s syndrome (3 of them had normal serum lipids and 3 normal serum bilirubin) Two patients had tran



Fig 2 Stomatocytosis as seen on a peripheral blood smear



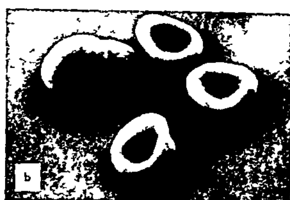




Fig 3 Scanning electron microscopy of erythrocytes from a normal individual (*a*) and from a patient with stomatocytosis (*b* and *c*)

sient stomatocytosis one of them however also had raised serum cholesterol and triglyceride levels and was classified as having a partial Zieve s syndrome as well Three patients had normal values for both bilirubin and lipids

DISCUSSION

When haemolysis is suspected in alcoholic patients the physician may be misled by laboratory data An increase in serum bilirubin lactate dehydrogenase or serum iron may be due to alcoholic liver disease rather than haemolysis A reduced serum haptoglobin level is usually taken to indicate haemolysis but may also result from decreased synthesis by a damaged liver Conversely haptoglobin as an acute phase protein" may be raised in inflammatory liver disease e g acute alcoholic hepatitis despite accompanying haemolysis Furthermore the reticulocytosis and erythroid hyperplasia often observed in alcoholic patients admitted to hospital rather than being interpreted as related to a haemolytic process may be signs of recovery from the suppressive effect of alcohol (8 11 12) Haemolysis may occur without compensatory erythroid hyperplasia when accompanied by bone marrow suppression as illustrated by patient 12 In order to establish the diagnosis of haemolysis in alcoholic patients erythrocyte survival studies are therefore usually required

With a normal bone marrow reserve one would expect to find the highest reticulocyte counts in patients with short erythrocyte half lives and low Hb levels The lack of obvious correlation between these parameters in our patients is probably due to a varying degree of concomitant bone marrow

suppression by ethanol Interestingly erythropoiesis seems to be at a maximum in the first bone marrow aspirate obtained before peripheral blood reticulocytosis reaches its summit (data not given) If this observation proves to be correct it may indicate the presence of ineffective erythropoiesis (intramedullary haemolysis) as long as the marrow is exposed to the effect of alcohol followed by bone marrow recovery manifested by peripheral reticulocytosis The shortened red cell half life recorded during abstinence from alcohol suggests that peripheral haemolysis continues for at least as long as this investigation lasted i e 2 weeks or more The present data are however insufficient to outline the particular features of alcohol induced haemolysis and further studies are needed

While the true incidence of ethanol induced haemolytic anaemia is unknown it is our impression that it is not rare Indeed mild shortening of the erythrocyte life span has been noted in a variety of hepatobiliary disease states including infective hepatitis obstructive jaundice and congenital hyperbilirubinaemia (21) Heck et al (10) have estimated the frequency of Zieve s syndrome in a general medical ward to be approximately one of 1600 admissions Interestingly the large male preponderance regularly found in that and in other reports (2 9) is not confirmed in our study Most of the studies on alcohol induced haemolytic anaemia have been performed on regular heavy drinkers or skid row alcoholics with chronic liver disease However 5 of our 14 patients were socially well adapted individuals without overt alcohol dependency and with only slight liver injury Thus in unexplained haemolysis as in the face of other puzzling haematological data (20) alcohol dependency must be specifically searched for

Attempts have been made to classify alcohol induced haemolytic anaemia into different categories Zieve (26) described haemolytic anaemia hyperbilirubinaemia and hyperlipidaemia in male alcoholics with moderate liver disease and he considered this a syndrome separate from the hypersplenism found in cirrhosis with portal hypertension and splenomegaly previously described by Jandl (13) Of our 14 patients 4 presented with all the characteristics of Zieve s syndrome In 6 patients however hyperbilirubinaemia and hyperlipidaemia occurred independently and 4 patients had haemolysis with normal values for both bilirubin and serum lipids Indeed both hyperbilirubinaemia and hyperlipidaemia are common findings in alcoholic patients (4 5 15) and their relation to haemolysis is obscure The features of Zieve s syndrome probably represent common and independent phenomena in alcoholic subjects even when occurring together The validity of Zieve s syndrome must thus be questioned

Douglass and Twomey (7) described acquired stomatocytosis with haemolysis in 4 male alcoholics with mild liver dysfunction and normal spleen size Two of our patients had a moderate increase in the number of stomatocytes in peripheral blood as assessed by microscopy of dry smears and by scanning electron microscopy However one of them also had hyperlipidaemia which was not a feature of the original syndrome Furthermore we have observed transient stomatocytosis often far more pronounced than in these 2 patients in alcoholics with liver disease who lack evidence of haemolysis (25) Recently acquired stomatocytosis has also been found in other non haemolytic disease states (6) Thus there seems to be no direct connection between this abnormal red cell morphology and haemolysis The stomatocytosis described in a rare type of congenital haemolytic anaemia in which the erythrocyte membrane is excessively permeable to cations (14) is probably not related to the kind of stomatocytosis noted in the patients in this study

The pathogenesis of ethanol induced haemolysis remains to be elucidated It is however possible that several factors such as erythrocyte membrane lability due to lipid abnormalities (24) or enzyme instability (9) may interact in susceptible individuals to tip the balance between erythrocyte stability and phagocytic cells in favour of haemolysis The influence of dietary habits vitamin intake and the level and duration of alcohol ingestion is possibly decisive but difficult to assess The relative importance of alcohol per se and the liver injury in causing haemolysis is unknown Although all the patients described by other authors (2 9 26) as well as those in the present report displayed some evidence of liver disease there is no correlation between the severity of liver affection and the degree of haemolysis

REFERENCES

1 Anderson T F Techniques for the preservation of three-dimensional structure in preparing specimens for the electron microscope Trans NY Acad Sci Ser III 13 130 1951

2 Balcerzak S P Westerman M P & Heinle E W Mechanism of anaemia in Zieve s syndrome Am J Med Sci 255 277 1968
3 Bessis M & Weed R I Preparation of red blood cells (RBC) for SEM A survey of various artefacts Proc ITT SEM 289 1972
4 Blass J P & Dean H M The relation of hyper lipemia to hemolytic anaemia in an alcoholic patient Am J Med 40 283 1966
5 Bottiger L E Carlson L A Hultman E & Romanus V Serum lipids in alcoholics Acta Med Scand 199 357 1976
6 Davidson R J How J & Lessels S Acquired stomatocytosis its prevalence and significance in routine haematology Scand J Haematol 19 47 1977
7 Douglass C C & Twomey J J Transient stoma tocytosis with hemolysis a previously unrecognized complication of alcoholism Ann Intern Med 72 159 1970
8 Eichner E R & Hillman R S The evolution of anaemia in alcoholic patients Am J Med 50 218 1971
9 Goebel K M Goebel F D Schubotz R & Schneider J Red cell metabolic and membrane fea tures in haemolytic anaemia of alcoholic liver disease (Zieve s syndrome) Br J Haematol 35 573 1977
10 Heck J Keitel K & Gehrmann G Zwischenbi lanz des Zieve Syndroms Dtsch Med Wochenschr 95 2058 1970
11 Hines J D & Cowan D H Studies on the pathogenesis of alcohol induced sideroblastic bone marrow abnormalities N Engl J Med 283 441 1970
12 Hourihane D OB & Weir D G Suppression of erythropoiesis by alcohol Br Med J 1 86 1970
Jandl J H The anaemia of liver disease observa tions on its mechanism J Clin Invest 34 390 1955
Lock S P Sephton Smith R & Hardisty R M Stomatocytosis a hereditary red cell anomaly as-sociated with haemolytic anaemia Br J Haematol 7 303 1961
15 Losowsky M S Jones D P Davidson C S & Lieber C S Studies of alcoholic hyperlipemia and its mechanism Am J Med 35 794 1963
16 Magnus E M Folate studies Folate and vitamin B12 values in relation to bone marrow pattern Scand J Haematol (Suppl) 24 70 1975
17 Matzkies F Hartwich G & Grabner W Das Zieve Syndrom Fortschr Med 90 390 1972
18 Mazia D Schatten G & Sale W Adhesion of cells to surfaces coated with polylysine J Cell Biol 66 198 1975
19 McCurdy P R Pierce L E & Rath C E Abnor mal bone marrow morphology in acute alcoholism N Engl J Med 266 505 1962
20 Myrhed M Berglund L & Bottiger L E Alcohol consumption and haematology Acta Med Scand 202 11 1977
21 Pitcher C S & Williams R Reduced red cell sur vival in jaundice and its relation to abnormal gluta-thione metabolism Clin Sci 24 239 1963
22 Smith J A Lonergan E T & Sterling K Spur cell anemia hemolytic anemia with red cells resem bling acanthocytes in alcoholic cirrhosis N Engl J Med 271 396 1964
23 Sullivan L W & Herbert V Suppression of hematopoiesis by ethanol J Clin Invest 43 2048 1964
24 Westerman M P Balcerzak S P & Heinle E W Red cell lipids in Zieve s syndrome their relation to hemolysis and to red cell osmotic fragility J Lab Clin Med 72 663 1968
25 Wisloff F & Boman D Unpublished observations
26 Zieve L Jaundice hyperlipemia and hemolytic anaemia a heretofore unrecognized syndrome as-sociated with alcoholic fatty liver and cirrhosis Ann Intern Med 48 471 1958

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Development of Biclonal Gammopathy in a Patient with von Recklinghausen's Neurofibromatosis

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ABSTRACT A well established case of von Recklinghausen's neurofibromatosis, in which a biclonal gammopathy developed, has been studied One of the paraproteins was characterized as IgG kappa the other as IgG lambda This combination of anomalies has not been described previously in the literature

Von Recklinghausen s neurofibromatosis is characterized by increased pigmentation of the skin (cafe au lait spots) and skin tumors partly of ectodermal and neural origin Mental deficiency is often present as are several organ manifestations including the skeleton eyes feet and blood vessels Hemangioma is often detected as well as hypertrichosis sacralis Axillary freckling has been emphasized as an important diagnostic aid (3) The pattern of inheritance is autosomally dominant (4)

An overall increase of secondary tumors has been observed in neurofibromatosis However to our knowledge no cases have been reported in the literature in which neurofibromatosis coexists with gammopathy or other disturbances in the immunoglobulin synthetizing apparatus In the present communication we report on a patient with monoclonal gammopathy who during two years observation developed biclonal gammopathy with IgG kappa and IgG lambda specificities Clinical evidence for the presence of myelomatosis has not been obtained It is suggested that an increased chance of somatic mutations might be present in neurofibromatosis

CASE REPORT

A 68-year-old man has noticed skin tumors melanotic skin macules and axillary freckling since the age of 35 No knowledge of similar defects in parents or siblings His son now aged 29 is disabled by a neurologic disease but he has refused any kind of physical examination For the last 2 years the patient has complained of dyspnea during excercise Physical examination demonstrated a rough systolic cardiac murmur with radiation to the carotides indicating a stenotic aortic valve A moderate hepatomegaly was disclosed Extensive Dupuytren s contractures were observed in both hands Otherwise the patient felt well No musculoskeletal tenderness back pain or other signs suggesting myelomatosis were demonstrated Skeletal X rays did not show any abnormalities Biopsy of excised tumor material verified the presence of neurofibromas Plasma cells were not detected in the biopsy Less than 2% plasma cells were demonstrated in his bone marrow Chromosome analysis demonstrating 46 XY karyotype did not reveal any abnormalities

Three months after the initial studies reported in this communication the patient suddenly died (mors subita) His sudden death can be related to the stenotic aortic valves

Analytical procedures

Agarose gel electrophoresis of serum proteins was carried out according to Johansson (7) Immunoelectrophoresis for demonstration of gamma alpha mu heavy chains and kappa and lambda light chains was performed conventionally on glass plates in 1% agarose gel The antisera were obtained from Boehringwerke Radial immunodiffusion was adopted from Mancini et al (10)

The size of the monoclonal components was evaluated after determination of total serum protein and specific scanning of individual components in a Gelman Automatic Scanner with Integrator

Light chains in urine were examined after concentration of morning samples 300 times in Amicon ultrafilters applying Heller s and Bradshaw s tests for protein In addition electrophoresis and immunoelectrophoresis of concentrated urine were carried out in agarose gel

Films from bone marrow aspirates were fixed in absolute methanol and stained by May-Grunwald-Giemsa at pH 6 8 for light microscopy

RESULTS

At the first examination (Oct 13th 1975) agarose gel electrophoresis demonstrated a monoclonal IgG kappa protein in the gamma region which made up approximately 20 g/l (Table I) Two years later (Oct 6th 1977) two monoclonal paraproteins were

Table I Development of biclonal components

Date of investigation	Type of M-component	
	IgG κ (g/l)	IgG λ (g/l)
Oct 13th 1975	20	0
Oct 6th 1977	15	7
Feb 15th 1978	16	8
March 10th 1978	20	7

detected in the gamma region the two components making up 15 g/l and 7 g/l respectively This result was verified by consecutive examinations (Feb 15th and March 10th 1978) In the last studies performed the two monoclonal proteins made up 20 g/l and 7 g/l respectively (Table I)

The monoclonal components were well separated from each other (Fig 1) Immunoelectrophoresis demonstrated that they contained heavy chains of gamma type (Fig 1) However one of the components contained lambda light chains (most cathodic location) while the other dominant monoclonal protein contained kappa light chains IgA and IgM were within the lower limit of the normal range (Table II)

Less than 2% plasma cells were detected in the bone marrow smear Serum calcium creatinine uric acid alkaline phosphatases ESR and Hb values were all within normal limits Light chains (Bence Jones protein) were not detected in urine

DISCUSSION

In occasional cases of multiple myeloma biclonal gammopathies have been reported Most often these are formed by IgG and IgA heavy chains (12) Other combinations including IgG and IgM have been reported In a large series of 870 IgG monoclonal proteins Skvaril et al (14) found that 12 had biclonal components Only one of these contained IgG paraproteins with different light chains (kappa and lambda) as in our patient Oriol et al (11) have reported on a case with two monoclonal proteins one with IgG_1 kappa and one with IgG_1 lambda specificities very similar to our observations but their case presented with overt clinical myelomatosis To our knowledge these two cases are the only ones to have been reported in the literature with this constellation of M-components

Detailed studies of Waldenstrom s macroglobulinemia have revealed biclonal patterns in a number of instances Harboe et al (6) reported that 14% of their patients had biclonal gammopathy Our patient however revealed no signs of macroglobulinemia

An extensive review of the clinical features of 30 patients with biclonal gammopathy disclosed that the patients may present with hyperviscosity syndrome amyloidosis polyarthritis malignant lymphoma solitary plasmocytoma multiple myeloma or macroglobulinemia (8) We have not been able to demonstrate any of these conditions in our patient In particular antinuclear factor Waaler s test and Ra Latex test were all normal as were AST ESR and Hb Neither have urinary light chains been detected nor an excessive number of plasma cells in the bone marrow The only primary

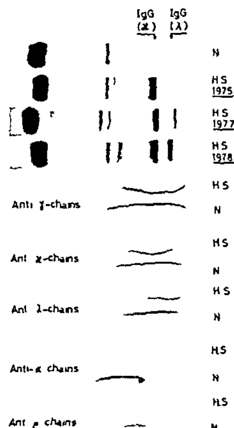


Fig 1 Agarose gel electrophoresis of normal (N) and patient serum (*H S*) in 1975 1977 and 1978 Note the development of biclonal gammopathy in the patient from a monoclonal protein of IgG kappa and possibly a faint cathodic extra band (1975) to a well developed biclonal gammopathy of IgG kappa and IgG lambda specificities in 1978

Table II *Serum immunoglobulins (g/l)*

	Patient s values	Reference values
IgG	40*	7 5–15
IgA	1 1	0 5–3 5
IgM	0 8	0 4–2 0

Discrepancy with the values obtained by scanning due to atypical IgG which give falsely increased values by radial immunodiffusion

disease detected apart from the valvular stenosis is neurofibromatosis associated with a certain mental deterioration

Monoclonal gammopathies have been reported in other neurologic diseases including myasthenia gravis (13) and ataxia telangiectasia (1) A patient with peripheral neuropathy and a small amount of monoclonal IgM protein in his serum was found to have infiltration of IgM producing lymphocytes in a saphenous nerve (5) However in none of these cases was biclonal gammopathy reported Gammopathies have also been reported in certain dermatological diseases Lergier and Gowans (9) demonstrated monoclonal gammopathy in a patient with psoriatic arthritis while Cream (2) reported on a case with pyoderma gangrenosum demonstrating a monoclonal IgM red cell agglomerating factor

The coexistence of von Recklinghausen s disease and biclonal gammopathy without myelomatosis has not been reported previously Whether the demostrated coexistence is of causal importance cannot be concluded from an isolated case Statistically the chance of obtaining such a combination is extremely small Very few cases of IgG biclonal gammopathy with different light chains have been reported in the literature (8)

An overrepresentation of other tumors in neurofibromatosis seems to be well established It seems to be possible that these multiple malignancies depend on an increased tendency to somatic mutations The development of two different monoclonal proteins in a patient who primarily disclosed one paraprotein and in whom the second paraprotein developed within 2 years could substantiate the hypothesis of somatic mutations

Despite the rather high amount of M components (more than 20 g/l) it is strange that the ESR is normal The disease seems to have a rather benign course No significant secretion of light chains has been demonstrated in the urine and the renal function as evaluated by creatinine values is still normal in contrast to that seen regularly in secretory myelomatosis No cytotoxic treatment has therefore been instituted The clinical significance of the observed association between von Recklinghausen s disease and defects in the immunoglobulin synthetizing apparatus must await further observation and more detailed studies which are in progress

REFERENCES

1 Cawley L P & Schenken J R Monoclonal hypergamma globulinemia of the gamma M type in a nine year old girl with ataxia teleangiectasia Am J Clin Pathol 54 790 1970

2 Cream J J Pyoderma gangrenosum with a monoclonal IgM red cell agglomerating factor Br J Dermatol 84 223 1971

3 Crowe F W Axillary freckling as a diagnostic aid in neurofibromatosis Ann Intern Med 61 1142 1964

4 Fenoan T T & Yakovac W C Neurofibromatosis in childhood J Pediatr 76 339 1970

5 Forssmann O Bjorkman G Hollender A & Englund N E IgM producing lymphocytes in peripheral nerve in a patient with benign monoclonal gammopathy Scand J Haematol 11 332 1973

6 Harboe M Hannestad K & Sletten K Oligoclonal macroglobulinemia Scand J Immunol 1 13 1972

7 Johansson B G Agarose gel electrophoresis Scand J Clin Lab Invest (Suppl) 124 7 1972

8 Kyle R A & Bayrd E D Biclonal gammopathies In The monoclonal gammopathies American lecture series pp 154–155 Thomas Springfield Ill 1976

9 Lergier J E & Gowans J D C Monoclonal IgM immunoglobulinemia in psoriatic arthritis JAMA 231 171 1975

10 Mancini G Vaerman J P Carbonara A O & Heremans J F A single radial diffusion method for the immunological quantitation of proteins In XI Colloquium on Protides of Biological Fluids (ed H Peters) pp 370–373 Elsevier Amsterdam 1964

11 Onol R Huerta J Bouvet J P & Liacpoulos P Two myeloma globulins IgG_1 kappa and IgG_1 lambda from a single patient I Purification and immunochemical characterization Immunology 27 1081 1974

12 Rosen B J Smith T W & Bloch K J Multiple myeloma associated with two serum M-components gamma G type kappa and gamma A type lambda N Engl J Med 277 902 1967

13 Rowland L P Ossermann E F Scharfmann W B Balsam R F & Ball S Myasthenia gravis with a myeloma type gamma G (IgG) immunoglobulin abnormality Am J Med 46 599 1969

14 Skvaril F Juncic D Spengler G A & Morell A The IgG subclass distribution in double M-component sera In Colloquium on Protides of Biological Fluids (ed H Peters) pp 273–277 Pergamon Press Oxford 1973

Peripheral Neuropathy in Monoclonal Gammapathy with Cryoglobulinemia and Arteritis

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ABSTRACT We report a patient with IgM gammapathy cryoglobulinemia Raynaud s phenomena purpura hyperglobulinemica of the legs and polyneuropathy Endoneural vasculitis with infiltrations of eosinophilic and neutrophilic granulocytes and an extensive loss or Wallerian degeneration of myelinated nerve fibers were seen on histopathologic examination of a sural nerve biopsy specimen The microscopic picture d ffered somewhat from that observed previously in cryoglobulinemic vasculitis Although vasculitis is most often believed to represent an immunologically mediated lesion we propose an alternate explanation namely that the disease manifestations in the present case were secondary to cold induced effects of the cryoglobulin on the microcirculation

Key wo ds neuropathy monoclonal gammapathy cryoglobul nem a arteritis

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Peripheral neuropathies are not infrequently seen in monoclonal gammapathies i e multiple myeloma and Waldenstrom s macroglobul nemia In the latter cond tion they are sa d to occur n 8–70% of the cases (4) The pathogenes s of these polyneuropa th es is poorly understood Amyloid depos tion or myeloma infiltrates have been observed in only a m nority of pat ents (5 9) In a recently reported case of Waldenstrom s macroglobulinem a and peripheral neuropathy attachment of the M component to nerve f bers could be demonstrated Vasculitis and cryoglobul nem a were not present (8)

In monoclonal gammapathies the M component may possess cryoprec p tating propert es S nce cryoprec pitat ng proteins may also be seen in many other disorders and may have vary ng composit on the following classif cat on has been proposed I A s ngle homogeneous prec p tate s present (monoclonal type) II Two or more immunoglobulins are found of which one is homogeneous (mixed type) III Two or more immunoglobulins are found none of wh ch is homogeneous (polyclonal type) (6)

Peripheral neuropath es have been reported in cryoglobulinem a with an incidence of 7 18% but in these investigations the cryoglobulins have not been classif ed systematically (3 7)

Nerve biopsy has been performed in a few cases of cryoglobulinemia and polyneuropathy and the histopathologic investigation revealed perivascular inf ltration of mononuclear inflammatory cells and demyelination or Wallerian degeneration of nerve fibers

We report histopathological immunological and b ochemical findings in a case of monoclonal IgM cryoglobulinemia

CASE REPORT

A 66-year-old man was admitted to the Department of Med c ne because of Raynaud s syndrome wh ch had developed during the preced ng two years He had been well unt l the occurrence of these symptoms wh ch were characterized by blanch ng of the f ngers of both hands on *exposure to cold W th n minutes the f ngers became* cyanot c and numbness was experienced During recovery the cyanos s was replaced by a bright red color and the numbness by throbb ng pa n t ngl ng and swell ng Troph c changes includ ng gangrene d d not develop No com pla nts eferable to peripheral neuropathy were p esent

On examinat on there was a m ld mpa rment to all modal t es of cutaneous sensat on n the feet V brat on sense was reduced below knees Jo nt pos tion was normal The other neurological and medical exam nat ons revealed normal f nd ngs On serum electrophores s an M-component clas f ed as IgM K was dent f ed at a con centration of 1? g/l Hb concentrat on and peripheral

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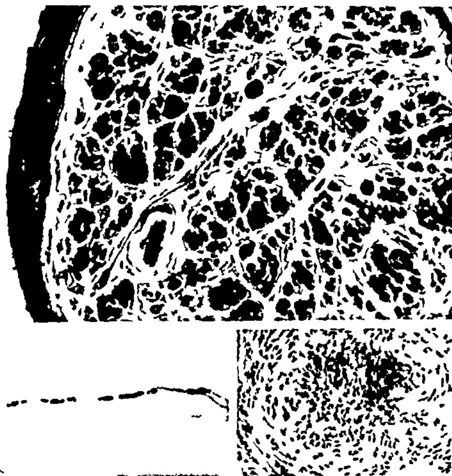


Fig 1 Sural nerve showing fibrinoid necrosis of epineural arteries and infiltration of the wall by polynuclear cells There is a severe loss of myelinated nerve fibers and in teased preparations there is Wallerian degeneration

blood counts were normal as was a microscopic examination of a bone marrow smear

During the following two years there was a progressive deterioration in addition to the disabling symptoms experienced on exposure to cold increasing numbness and paresthesia in the feet occurred The latter symptoms were not accompanied by pain During this time the patient had also noticed purpuric spots on the lower legs The spots recurred frequently and were succeeded within n days by brownish patches

Somatic examination revealed numerous skin lesions about 3×3 mm in diameter with brown pigmentation and dermal atrophy in the lower legs but no ulcerations The peripheral pulsations were normal Raynaud s phenomena as described above could be reproduced by immersion of his hands in water at 15 C The ankle reflexes were absent and the other neurological findings were unchanged

Immunoelectrophoresis of serum showed an IgM K component in a concentration of 14 g/l (reference value 0 05–?) The other estimated serum protein fractions were within the normal range albumin 41 g/l haptoglobin 1 3 g/l IgG 13 8 g/l IgA 1 4 g/l complement C_3 100% and complement C_4 60%

A cryoglobulin test was strongly positive showing an estimated cryoglobulin concentration of ? 3 g/l At repeated serum electrophoresis after cryoprecipitation the IgM component was reduced correspondingly while the IgG and IgA concentrations were unchanged A reagin test for syphilis was negative HB_sAg could not be demonstrated The rheumatoid factor activity and antinuclear activity were also negative Cryoagglutinins and cryofibrinogen were not present

The CSF protein concentration was 0 46 g/l Electrophoresis of the CSF showed a pattern consistent with blood–brain barrier damage and there was an M component of the same mobility as in serum A CSF cell count was normal Urine electrophoresis was unrevealing, i e no light chains could be demonstrated

The Hb concentration was 14? g/l WBC 7 4×10[9] with a differential count as follows 0% neutrophilic band forms 76% segmented neutrophils 14 5% lymphocytes 6% monocytes 3% eosinophils and 0 5 % basophils The platelet count was 171×10[9] Microscopic examination of the bone marrow showed a moderate increase in plasma cells some of which were abnormal and a slight increase in lymphoid cells

Neurophysiological studies disclosed that motor nerve

conduction velocity was reduced symmetrically in the median nerves and no response was obtained in the peroneal nerves A needle electrode examination of m extensor dig brevis bilaterally was interpreted as compatible with moderate polyneuropathy Microscopic examination of a specimen from m tibialis anterior revealed degeneration of muscle fibers as seen in peripheral neurogenic lesions

At microscopical investigation of a biopsy specimen from the sural nerve several epineural arteries showed fibrinoid necrosis and infiltration of the wall by several eosinophilic granulocytes some neutrophilic granulocytes lymphocytes and macrophages There was a severe loss of myelinated nerve fibers and several of the remaining ones showed Wallerian degeneration in teased preparations No signs of primary demyelination or amyloid deposition were seen (Fig 1)

Treatment was started with busulphan prednisolone penicillamine and repeated plasmapheresis No progress of the symptoms has occurred and the neurological findings were unchanged one year after the initiation of therapy Examination of the bone marrow yielded a slight increase in morphologically normal plasma cells but the number of lymphoid cells was normal The IgM component was 7 g/l i e about 50% of the pretreatment concentration

DISCUSSION

The cryoglobulin in our patient was probably monoclonal Regardless of its type the histopathology differs from that previously described in cryoglobulinemic vasculitis the picture showing some similarities to periarteritis nodosa The underlying plasma cell dyscrasia may represent an early stage of Waldenstrom s macroglobulinemia

An intriguing question is the pathogenetic relation between the cryoglobulin and the neuropathy The simultaneous development of purpura hyperglobulinemica and neuropathy with vasculitis in epineural arteries suggests that both these manifestations could be the result of a compromised microcirculation The vasculitis seen in experimental serum sickness has been regarded as a model for the various clinical conditions in arteritis (2) Accordingly this may be true in many instances but it must be born in mind that the term vasculitis is only descriptive and does not per se imply an immunologic mechanism

In our patient we cannot exclude an immunologic mechanism but evidence such as the presence of rheumatoid factor antinuclear antibodies and signs of complement activation is lacking Furthermore the occurrence of Raynaud s syndrome may indicate an alternative non immunologic explanation It would thus seem more consistent to regard the Raynaud s phenomena as reversible and the skin lesions and neuropathy as irreversible results of the cold induced effects of the cryoglobulin on the microcirculation Autoantibodies and signs of complement activation are frequently found in mixed cryoglobulinemia (1) On the contrary such findings are rare in monoclonal gammapathy (10) Also the histopathological picture in our patient with fibrinoid necrosis and infiltration of eosinophilic and neurophilic granulocytes differs from that previously reported in cryoglobulinemic vasculitis and polyneuropathy Furthermore it does not seem reasonable to expect an immunological disorder to spare the upper extremities

Present support for various specific relations between monoclonal gammapathies and polyneuropathy may however justify the recommendation that electrophoretic screening procedures should be carried out in cases exhibiting peripheral neuropathy

REFERENCES

1 Brouet J C Clauvel J P Danon F Klein M & Seligmann M Biologic and clinical significance of cryoglobulins Am J Med 57 775 1974

2 Christian C L & Sergent J S Vasculitis syndromes Clinical and experimental models Am J Med 61 385 1976

3 Cream J J Hern J E C Hughes R A C & MacKenzie I C K Mixed or immune complex cryoglobulinemia and neuropathy J Neurol Neurosurg Psychiatry 37 82 1974

4 Dayan A D & Lewis P D Demyelinating neuropathy in macroglobulinemia Neurology (Minneap) 16 1141 1966

5 Dayan A' D Urich H & Gardner Thorpe C Peripheral neuropathy and myeloma J Neurol Sci 14 21 1971

6 Grey H M & Kohler P F Cryoimmunoglobulins Semin Hematol 2 87 1973

7 Logothetis J Kennedy W R Ellington A & Williams R C Cryoglobulinemic neuropathy Arch Neurol 19 389 1968

8 Propp R P Means E Deibel R Cherer G & Barron K Waldenstrom s macroglobulinemia and neuropathy Neurology (Minneap) 25 980 1975

9 Trotter J L Engel W K & Ignazak T F Amyloidosis with plasma cell dyscrasia Arch Neurol 34 209 1977

10 Waldenstrom J Diagnosis and treatment of multiple myeloma Grune & Stratton New York 1970

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Ectopic Atrial Tachycardia on Swallowing

Report on Favourable Effect of Verapamil

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ABSTRACT A female patient who suffered from atrial tachycardia associated with the ingestion of food or drink was examined in our department No signs of organic heart disease were discovered, oesophageal motility was normal but X ray revealed a small hiatal hernia The arrhythmia started with an atrial extrasystole arising well outside the functional refractory period of the AV node, and it could be reproduced by inflation of a balloon It is suggested that the arrhythmia is induced by a mechanical effect of the passage of food on the left atrial wall Several drugs were tried in order to stop or relieve the complaints None of them prevented or stopped the atrial tachycardia but verapamil and edrophonium chloride caused 2 1 AV block, and follow up study has shown that sufficient doses of verapamil are able to relieve the patient s complaints

Key words ectopic atrial tachycardia swallowing verapamil

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Cardiac arrhythmias on swallowing are rare disorders Various degrees of AV block nodal or sinus bradycardia sinus arrest ventricular asystole and atrial fibrillation have been reported (10 11) Swallowing syncope has been associated with functional and organic diseases of the oesophagus (1 11) and the disturbances in cardiac rhythm have been thought to be due to the effect of the cardiac conducting system of a vagovagal reflex with the afferent impulses from the oesophagus and the efferent discharges producing various arrhythmias (10)

We here report a patient who suffered from atrial tachycardia associated with the ingestion of food or drink

CASE REPORT

A 61 year-old woman was seen in Jan 1975 in our department with several years history of palpitations on swallowing expecially after solid food These symptoms were sometimes associated with dizziness Before admission she had tried digitalis quinidine and propranolol (no information on the doses) without any significant relief though verapamil had been of some value The duration of the palpitations was short and only related to the passage of food There was no history of organic heart disease the BP was normal and the pulse regular

The results of physical examination were normal the ECG showed regular sinus rhythm and haematological and urinary examinations revealed no abnormalities A chest X ray showed no cardiac enlargement X ray of oesophagus revealed a small hiatal hernia the motility was normal The sinus node recovery time was normal and His bundle electrogram showed normal PH and HQ times On swallowing the arrhythmia was initiated by an atrial extrasystole appearing 0 48 sec after the last normal P wave (Fig 1 Table I) The first atrial extrasystole was conducted to the ventricles with a normal PQ time (0 20 sec) During tachycardia however AV nodal conduction time increased to 0 22 sec (Fig 1 Table I) The P_T–P_T interval during tachycardia was 0 40 sec and the tachycardia was unblocked 1 1 AV conduction A balloon (Sengstaken s tube) inflated to a pressure of 35 mm of mercury approximately 30 cm from the orifice of the mouth reproduced the tachycardia

Several drugs were tried in order to prevent stop or relieve her complaints Verapamil and Tensilon (edrophonium chloride a cholinesterase inhibitor) caused 2 1 AV block whereas disopyramide and atropine exerted no effect on AV nodal conduction (Fig 2 Table I) Practolol caused partial 2 1 AV block (Table I) None of the drugs prevented or stopped the atrial tachycardia Verapamil Tensilon practolol and to a lesser extent disopyramide increased AV nodal conduction time during tachycardia (Table I)

Abbreviations AV=atrioventricular P_s–P_E=interval (sec) between the last sinus beat and the first atrial extrasystole P_T–P_T=interval (sec) between atrial beats during atrial tachycardia R_T–R_T=interval (sec) between ven[illegible] beats during atrial tachycardia BP=blood [illegible] ECG=electrocardiogram

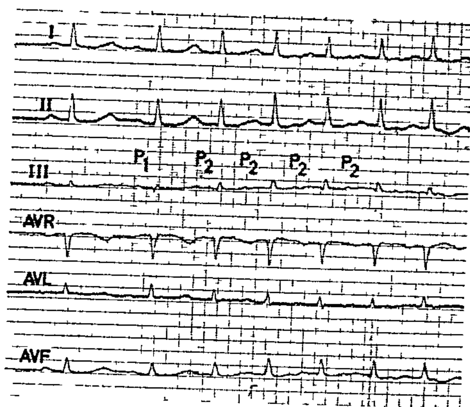


Fig. 1. Atrial tachycardia on swallowing. P_1 = P waves during sinus rhythm. P_2 = P waves during atrial tachycardia. Paper speed 50 mm/sec.

DISCUSSION

Various cardiac arrhythmias have previously been described to be associated with functional organic sorders of the oesophagus. The oesophageal ility in our patient, however, was normal, but ray revealed a small hiatal hernia. Atrial tachycardia has, as far as we know, not been reported earlier in connection with swallowing. The arrhythmia could be reproduced by inflation of a balloon.

The arrhythmia always started with an atrial extrasystole arising approximately 0.48–0.56 sec after the last normal sinus beat, i.e. the premature depolarization initiating the tachycardia arose well outside the functional refractory period of the AV node (2), and the PQ interval was not longer than that of the preceding sinus beat. This indicates that the arrhythmia occurring in this patient was an atrial ectopic tachycardia, perhaps induced by a mechanical effect of the passage of food on the left atrial wall. The fact that atropine did not prevent the tachycardia favours the view that a vagovagal reflex mechanism was not involved in the initiation of the arrhythmia. Paroxysmal supraventricular tachycardia starts when a single atrial premature depolarization enters the AV node during a specific

Table I. *AV nodal conduction times (sec) during sinus rhythm (P_1Q_1) and during atrial tachycardia (P_2Q_2)*

	PQ time	P_1–P_2 interval	P_2–P_2 interval	R_2–R_2 interval	P_2Q_2 time
No drug	0.20	0.48	0.40	0.40	0.22
Verapamil 8 mg i.v.	0.20	0.50	0.32	0.64	0.28
Tensilon 10 mg i.v.	0.20	0.52	0.32	0.64	0.28
Practolol 7 mg i.v.	0.20	0.56	0.45	0.45*	0.24
Disopyramide 100 mg×3 (2 days)	0.20	0.56	0.46	0.46	0.24
Atropine 0.8 mg i.v.	0.18	0.50	0.37	0.37	0.18

* Atrial tachycardia partially blocked

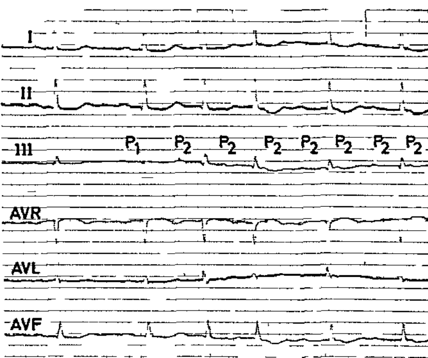


Fig 2 Effect of verapamil on atrial tachycardia on swallowing P_1 and P_2 as in Fig 1 Paper speed 50 mm/sec

portion of its functional refractory period (5 6) and the subsequent PQ interval is therefore always prolonged This allows the impulse to reenter the atrium where it may once again enter the AV node during its functional refractory period—the cycle is repeated and supraventricular tachycardia is the result (4 7) Pharmacological agents that increase AV nodal refractoriness and decrease AV nodal conduction velocity (verapamil Tensilon and some β adrenergic blockers) may terminate and prevent paroxysmal supraventricular tachycardia (3 13 16) In our patient verapamil and Tensilon did not prevent or stop atrial tachycardia though these drugs did have an effect on the AV node producing 2 1 AV block In contrast to propranolol even very high doses of practolol (0 2 mg/kg b wt) do not have any direct depressant effect on the AV node in 10 patients with ischaemic heart disease showing no signs of conduction disturbances or cardiac failure (15) This is probably the explanation why this drug only partially blocked the atrial tachycardia in the present case It is possible that the effect of practolol on the AV nodal refractoriness is minimal because of the drug s sympathomimetic activity (14) Disopyramide is a new antiarrhythmic drug with a quinidine like effect (12) It has been shown that it may reduce the incidence of atrial and especially ventricular arrhythmias (8) In the present study the drug neither prevented the occurrence of atrial tachycardia nor influenced AV nodal conduction It has previously been demonstrated that the effect of this drug on the AV node is minimal or variable (8 9)

The drug of choice in our patient proved to be verapamil It is a safe drug it is easy to administer and follow up study has shown that sufficient doses (40 mg×6) are able to relieve her complaints

REFERENCES

1 Alstrup P & Pedersen S A A case of syncope on swallowing secondary to diffuse oesophageal spasm Acta Med Scand 193 365 1973
2 Bissett J K de Soyza N Kane J J & Murphy M L Atrioventricular conduction patterns in patients with paroxysmal supraventricular tachycardia Am Heart J 91 287 1976
3 Cantwell J D Dawson J E & Fletcher G F Supraventricular tachyarrhythmias Treatment with edrophonium Arch Intern Med 130 221 1972
4 Gettes L S & Yoshonis K F Rapidly recurring supraventricular tachycardia A manifestation of reciprocating tachycardia and an indication for propranolol therapy Circulation 41 689 1970

5 Goldreyer B N Intracardiac electrocardiography in the analysis and understanding of cardiac arrhyth mias Ann Intern Med 77 117 1972
6 Goldreyer B N & Bigger J T Jr Spontaneous and induced reentrant tachycardia Ann Intern Med 70 97 1969
7 Han J The mechanism of paroxysmal atrial tachy cardia Sustained reciprocation Am J Cardiol 26 329 1970
8 Jennings G Model D S Jones M B S Turner P P *Besterman E M M & Kidner P H Oral* disopyramide in prophylaxis of arrhythmias following myocardial infarction Lancet 1 51 1976
9 Jensen G Sigurd B & Uhrenholt A Hae modynamic effects of intravenous disopyramide in heart failure Eur J Clin Pharmacol 8 167 1975
10 Kalloor G J Singh S P & Collins J L Cardiac arrhythmias on swallowing Am Heart J 93 235 1977
11 Kopald H H Roth H P Fleshler B & Pritchard W H Vagovagal syncope Report of a case as sociated with diffuse esophageal spasm N Engl J Med 271 1238 1964
12 Landmark K Disopyramid Et nytt antiarytmikum Tidsskr Nor Laegeforen 31 1613 1977
13 Landmark K & Amlie J P A study of the verapamil induced changes in conductivity and re fractoriness and monophasic action potentials of the dog heart in situ Eur J Cardiol 4/4 419 1976
14 Refsum H & Landmark K The action of practolol on the isolated rat atrium Acta Pharmacol Toxicol 31 97 1972
15 Smithen C S Balion R & Sowton E Use of His potentials to assess changes in atrioventricular con duction produced by a series of beta adrenergic block ing agents Br Heart J 33 955 1971
16 Wu D Denes P Dhingra R Khan A & Rosen K M The effects of propranolol on induction of AV nodal reentrant paroxysmal tachycardia Circulation 50 665 1974

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Association of Pancreas Affection and Yersiniosis

A Case Report

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ABSTRACT A case history of a 25-year old female is reported She had no diseases predisposing for pancreatic disease A few days after a familial outbreak of yersinia enteritis she again became ill and was referred to hospital Clinical and laboratory examination made it likely that she underwent an acute pancreatic affection and the serological test proved that she had been through an acute yersiniosis The coincidental occurrence may of course be by chance, but considering the immunological aspects of secondary yersiniosis there might also be an etiological link

Key words pancreas affections yersiniosis

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Human pathogenicity of yersinia enterocolitica was discovered in 1948 Different aspects of yersiniosis are given among others by Ahvonen (3) and Winblad (20) In human beings the primary infection is *usually localized to the distal part of the small bowel* giving clinical pictures of various acute abdominal diseases (7 15 18) although sepsis and other manifestations have also been reported (9 10 17) A bacteriological culture is often positive in this phase The secondary phase is dominated by symptoms from skin joint or muscular systems (6 11 14 21 22) though involvement of heart (5) eyes (4) nervous system (2) kidneys (16) liver (8 16) and pancreas (19) has been reported In this phase bacteriological cultures are usually negative and the diagnosis is based upon serological tests

CASE HISTORY

A 25 year-old Caucasian woman with no previous major diseases She took no other drugs than contraceptive pills and seldom used alcohol Two weeks before admission she and her family had had an enteritis lasting for four days Serological tests performed later suggested that this had been an outbreak of yersinia infection Three days before admission she became acutely ill with increasing abdominal pain fever nausea and vomiting

On admission the patient was slightly deteriorated and her abdomen was tender upon palpation with a distinct maximum in the left upper quadrant Laboratory investigations S amylase 880 U/l (upper normal limit 300) with an increase on the next day to 6420 U/l ESR 70 mm/h Hb 10 g/100 ml She was treated with absolute diet except for intake of an anticholinergic drug and an antacid She improved in a few days S amylase normalized gradually during the 14 days in hospital ESR fell to 14 mm/h and Hb rose to 12 8 mg/100 ml Further investigation gave no signs of liver renal or cardiac affection She had neither skin lesions nor any joint pains Blood glucose calcium phosphorus and lipids were normal and fecal cultures negative

Later i v cholangiograms endoscopic pancreatic ductography and glucose tolerance test were all normal Yersinia enterocolitica titer against serogroup 3 was 1/1250 falling to 1/320 after 10 weeks Titer determinations were also carried out in the patient s family members one week and ten weeks after her admission to hospital showing values of 1/5000 and 1/650 for her husband 1/1250 and 1/320 for one child Only the titer at ten weeks (1/160) was available from her other child

Other serological tests (all performed at Statens Institutt for Folkhelse Oslo) gave no sign of other infective diseases Later the patient has been healthy and has taken her pill She does not belong to tissue type HLA B27

DISCUSSION

We interpreted the abdominal disease of our patient during the hospital stay as an acute pancreatic affection She was no alcoholic abuser and did not have any signs of liver or biliary disease in contrast to what was reported by Petterson and Gordin (19) We could not find other etiological explanations for her panatic affection than yersiniosis

The nature of the secondary phase of yersiniosis remains as obscure as the occurrence of similar clinical pictures in infections with other enteropathogens Theories concerning immunological response to the lipopolysaccharide content at the bacterial wall have been discussed (12 13)

Differences in organ manifestations are not well

understood except for arthritis where a genetic disposition is possible Patients with yersinia arthritis seem to have the same overrepresentation of about 90% HLA B27 positivity (1), like patients with Bechterew s disease psoriasis arthritis and other reactive arthritis The incidence of HLA B27 in yersiniosis without arthritis is similar (about 15%) to that of the normal population We feel we have encountered an association of acute pancreatic affection and secondary yersiniosis Whether this association is etiological must remain an open question that points to the old problem of the pancreas in immunological diseases

REFERENCES

1 Aho K Ahvonen P Lassus A Sievers K & Tiilikainen A Yersinia arthritis and related diseases clinical and immunogenetic implications In Infections and immunology in the rheumatic diseases WHO/ARC int symp London 1974 (ed D C Dumonde) pp 341–344 Blackwell London 1975
2 Ahvonen P Ann Clin Res 4 39 1972
3 — Studies on human yersiniosis in Finland Acad diss 1–7 Helsinki 1972
4 Ahvonen P & Dickhoff K Acta Ophthalmol (Suppl) 123 209 1974
5 Ahvonen P Hiisi Brummer L & Aho K Ann Clin Res 3 69 1971
6 Ahvonen P Sievers K & Aho K Acta Rheumatol Scand 15 232 1969
7 Andersen L Christiansen P M Lauritzen K B Korner B Jepsen O B Henrichsen S Brenø E Hancke A B & Johansen A Ugeskr Laeger 139 71 1977
8 Bakken A F & Blichfeldt P Scand J Rheumatol 5 174 1976
9 Bliddal I & Kaliszan S Acta Med Scand 201 387 1977
10 Gutman L Ottesen E A Quan T J Noce P S & Katz S L N Engl J Med 288 1372 1973
11 Hannuskela M & Ahvonen P Scand J Infect Dis 1 17 1969
12 Kanamori M Jpn J Microbiol 20 273 1976
13 Larsen J H Ugeskr Laeger 138 538 1976
14 — Yersinia enterocolitica infections and arthritis In Infections and immunology in the rheumatic diseases WHO/ARC int symp London 1974 (ed D C Dumonde) pp 133–139 Blackwell London 1975
15 Lassen J Tidsskr Nor Laegeforen 92 567 1972
16 Leino R & Kalliomaki J L Ann Intern Med 81 458 1974
17 Mollaret H H Pathol Biol 19 189 1971
18 Nilehn B & Sjöström B Acta Pathol Microbiol Scand 71 612 1967
19 Pettersson T & Gordin R Ann Clin Res 2 157 1970
20 Winblad S Yersiniainfektioner hos människan Medicinsk årsbok 1970 pp 254–258 Munksgaard Copenhagen 1970
21 — Scand J Infect Dis 1 11 1969
22 — Scand J Infect Dis 7 191 1975

Temperature Regulation in Anorexia Nervosa Patients during Prolonged Exercise

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ABSTRACT The thermal responses to prolonged exercise on a stationary bicycle ergometer have been studied in 10 anorexia nervosa (AN) patients and 5 normal subjects The patients were young (12–18 years) females (except for 1 case) and had less than 10% of their body weight as fat The basic experiments were conducted in a moderate environment (24 C) at approximately 65% maximal aerobic power output on all patients and controls In addition 2 patients were studied in warm (32 C) conditions 1 patient in cool (12 C) conditions and 2 patients after being heated for 30 min in a sauna at a temperature of approximately 50 C Measurements included oxygen uptake metabolic (M) and total heat production evaporative sweat loss (E) rectal (T) and mean skin temperatures The results showed that T_{re} (for a given M) was higher and E lower in the patients than the controls Passively heating the anorexia patients before exercise increased resting T_{re} and E but did not affect the plateau value of T_{re} obtained at the end of work In the warm environment T_{re} rose to the same level as observed at 24 C but did not rise above the resting value in cool conditions Thus it would appear that although patients with AN can regulate their body temperature adequately in a moderate environment loss of body fat which reduces thermal insulation may decrease the range of ambient temperatures over which T_{re} can be maintained during exercise Our data do not support the theory of a loss of central (hypothalamic) thermoregulatory control in anorexia

Key words anorexia nervosa temperature regulation prolonged exercise rectal temperature skin temperature sweat loss

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Primary anorexia nervosa (AN) is universally characterized by weight loss due to a failure to eat (3 5) The physical features of AN are those of chronic inanition the patients are often extremely emaciated with pallid and sallow skin but rarely show signs of vitamin iron or protein deficiency diseases One of the major physical concerns of the patients is a feeling of cold particularly in the extremities Their hands and feet even in a thermally neutral environment are vasoconstricted acrocyanosed and give rise to a feeling of numbness and sometimes pain It is surprising therefore that although low body temperatures and hypothermia (22) have often been reported in AN few attempts have been made to study thermoregulation in this disease Wakeling and Russel (21) have reported the effects of a localized peripheral heat stress induced by placing the arm up to the elbow in water at 45 C on oral and finger temperatures in 11 anorexic girls Gleeson and Moore (10) have studied the effects of mild heat and Mecklenburg et al (14) of cold stress in a climatic chamber on 5 anorexia nervosa patients

To our knowledge no attempt has been made to study the thermoregulatory responses to a given metabolic heat load resulting from exercise in AN The present investigation examines metabolic and thermal responses to prolonged work in 10 patients with AN and 5 healthy normal subjects

SUBJECTS AND METHODS

The patients formed part of a larger study (8 9) They conformed to the following criteria for AN chiefly accord

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Abbreviations AN anorexia nervosa $\dot V_{O_2}$ oxygen uptake $\dot V_{O_2}$ max maximal aerobic power T_{re} = rectal temperature T_{sk} = skin temperature $\bar T$ = mean temperature M metabolic heat production H = total heat production E evaporative sweat loss S heat storage — T_{db} dry temperature T_{wb} wet temperature C convection R = radiation

Table I *Physical characteristics of the patients and controls (mean ±1 S D)*

Ht=height Wt=weight LBM=lean body mass

	Age (y)	Ht (cm)	Wt (kg)	LBM (kg)	V_{O_2} max (l/min)
Patients (*n*=10)	15 0 ±2 0	164 ±15	37 9 ±8 1	34 1 ±7 2	1 18 ±0 30
Controls (*n*=5)	14 9 ±3 1	166 ±14	48 9 ±12 9		2 30 ±0 47

ing to Dally (5) 1) Age at onset less than 25 years 2) Active refusal to eat with accompanying pronounced weight loss 3) No evidence of schizophrenia severe depression or organic diseases

The physical characteristics of the patients with AN and the healthy controls are given in Table I The anorexic state of the patients had lasted on average for 1 0 year (range 0 5–3) prior to the investigation The normal menstruation and ovulatory cycles had ceased in the female patients at the time of measurement They were studied at rest and during work on an electrically braked bicycle ergometer at approximately 65% of their maximal aerobic power (V_{O_2} max) for 1 hour The subjects were weighed nude before and immediately after exercise on a balance accurate to ±10 g. Rectal temperature (T_{re}) was measured during exercise from a thermocouple inserted into the rectum 8 cm above the internal sphincter Skin temperature (T_{sk}) was measured at 14 sites finger and thumb hand upper and lower arm forehead pectoral sternum abdomen scapular lumbar anterior and posterior thigh and anterior and posterior calf at rest and during the 2nd 8th 20th 30th 40th 50th and 60th min of exercise using a thermistor probe mounted on an applicator The recordings (except finger and thumb measurement) were weighted after the method of Hardy and Dubois (11) and the average was taken as the mean body skin temperature ($\bar{T}_{sk}$) Oxygen uptake (V_{O_2}) was measured at rest and during the 28–30th and 58–60th min of exercise by the open circuit (Douglas bag) technique O_2 and CO_2 content of expired air being determined by the micro-Scholander technique Heart rate was calculated from standard ECG recordings at rest and every 2 min during exercise From the raw data calculations of metabolic (*M*) and total heat (*H*) production evaporative sweat loss (*E*) and heat storage (*S*) were made using standard equations (15) Dry (T_{db}) and wet (T_{wb}) temperatures in the laboratory were *23 6±0 84 and 14 1±2 2°C respectively In two patients* the experiments were repeated with and without preheating For this purpose the patients sat in a sauna for 30 min at about 50°C T_{db} and 26 C T_{wb} prior to exercise In addition two patients performed exercise at an elevated environmental temperature (T_{db} 32 2±0 4 T_{wb}=17 6±1 40 C) and one patient worked in a cool environment (T_{db} 11 7±0 4 T_{wb} 6 4±0 5°C) Control experiments were conducted on 4 healthy boys and one healthy girl (Table I) in the laboratory and 2 healthy boys in the cool and warm environments

V_{O_2} max was measured in the patients and control subjects on separate occasions For reasons previously given and discussed (8) it was impossible to establish with abso-

Table II *Thermoregulatory responses to exercise of AN patients and controls (mean ±S D)*

	V_{O_2} (l/min)	V_{O_2} max (%)	Load (W)	*M* (W)	*H* (W)	T_{re}[a] (°C)	$\bar{T}_{sk}$[a] (°C)	T_{re}[b] (°C)	$\bar{T}_{sk}$[b] (°C)	*E* (W)
12°C										
Patients (*n*=1)	1 31	73	90	447	357	36 58	30 09	36 80	29 10	3
Controls (*n*=2)	1 88	58	140	641	508	37 10	30 98	38 40	28 94	153
24°C										
Patients (*n*=10)	0 78 ±0 20	66 ±10	54 ±17	270 ±66	215 ±50	36 60 ±0 56	31 68 ±0 73	38 07 ±0 51	32 18 ±1 15	54 ±35
Controls (*n*=5)	1 61 ±0 39	64 ±8	116 ±32	548 * ±132	432 ±100	37 22 ±0 19	32 11 ±0 64	38 56 ±0 36	31 30 ±0 61	467 * ±69
24°C with preheating										
Patients (*n*=2)	1 07	68	80	363	298	36 75	33 43	38 40	35 10	117
32°C										
Patients (*n*=2)	0 91	56	75	311	236	36 55	33 19	38 25	35 84	146
Controls (*n*=2)	1 78	62	123	607	484	37 70	33 75	38 40	33 46	415

* $p<0.001$ ** $p<0.01$

[a] At rest [b] At 60th min of exercise

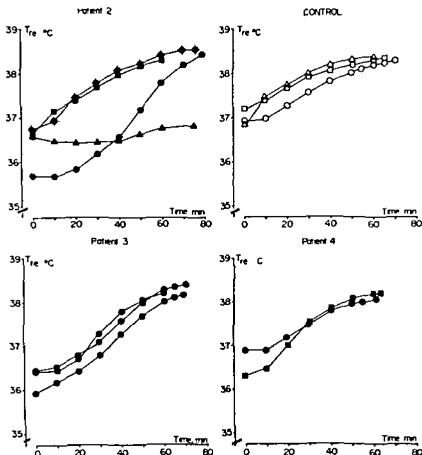


Fig 1 Time course of T_{re} changes during exercise in 3 AN patients and 1 control subject at 12 C (▲ △) 24 C with (●) and without (● ○) preheating and at 32 C (■ □) Patient 2 developed signs of heat pyrexia at T_{re} 32 C and the exercise was terminated It should be noted that the absolute level of T_{re} reached at the end of exercise in the patients was unrelated to the initial value and the change in body heat stores (cf Bittel & Henane (4)) produced by preheating

lute certainty the accepted criterion of $\dot{V}_{O_2}$ max namely a plateau of $\dot{V}_{O_2}$ with increasing work load (6) Secondary criteria based on the work of Åstrand (1) were therefore used in this study Evidence of maximal effort was accepted only if blood lactate exceeded 9 mmol/l and the respiratory exchange ratio was higher than unity This was achieved in all patients and controls Lean body mass was calculated from the sum of ten skinfold thicknesses after the method of Pařízková (17)

RESULTS

The metabolic and thermal responses to exercise of the anorexic patients and control subjects are given in Table II Fig 1 shows the typical T_{re} changes with exercise in AN in cool moderate (with and without preheating) and warm environments In moderate conditions without preheating the T_{re} of the patient increases slowly to reach a delayed plateau at 50th–80th min of exercise Preheating raises the resting T_{re} but has little effect on the rate of change and the final plateau level of T_{re} during exercise Raising the ambient temperature to 32 C during the whole work period has the same effect the onset of the T_{re} rise was more rapid but the rate of change and final value of T during exercise are similar to those found in the moderate environ

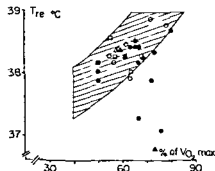


Fig 2 Relationship of T_{re} to % $\dot{V}_{O_2}$ max The shaded area shows the limits previously found for sedentary healthy subjects (7) Symbols as in Fig 1

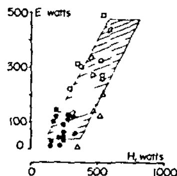


Fig 3 E corrected for metabolic and respiratory water losses in relation to H during exercise Symbols and shaded area as in Figs 1 and 2

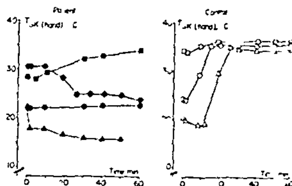


Fig 4 Hand temperatures of an AN patient and a control subject exercising at 3 different environmental temperatures Symbols as in Fig 1

ment When the ambient temperature was decreased to 12°C however T_{re} did not rise above resting condition and remained at an almost constant level through exercise

In the control experiments the rate of change and final plateau level of T_{re} were unaffected by ambient temperature over the range studied (Fig 1 and Table II) At 24 and 32°C T_{re} of patients and controls was related to M but more closely associated with the relative metabolic heat stress resulting from exercise (Fig 2) The relationship of T_{re} to % V_{O_2}max in 7 patients was similar to that found previously in healthy normal adults (7) but the points in 3 patients were below expected limits for the two variables (Fig 2)

Absolute E was lower in the patients than in controls (Table II) but for a given H E lay within the expected levels for normal subjects (Fig 3) At 24 C the patients stored 10% of their H and dissipated 25% by evaporative and 65% by non evaporative channels The corresponding figures for controls were 7 62 and 21% respectively Heat loss from the lungs corresponded to 9% of H in both groups Preheating increased E by approximately 11% and decreased S to approximately 2% in two patients

At the end of exercise at 24 C T_{sk} was 0 88°C higher in the anorexic patients than in controls preheating in the two patients had little effect on their final $\bar{T}_{sk}$ values Increasing environmental temperature to 32 C raised E and $\bar{T}_{sk}$ and reduced the amount of heat dissipated by convection (C) and radiation (R) in both groups (Table II) However although T_{sk} was higher in the anorexic patients at both environmental temperatures there were distinct differences in the pattern of T_{sk} response to exercise in different parts of the body particularly the extremities For example (Fig 4) the hands of the anorexic patients tended to remain cold and vasoconstricted even following preheating in moderate environment despite the fact that their T_{sk} remained higher than the controls Only when the patients exercised under warm conditions did the blood vessels of the extremities become dilated and T_{sk} of the hand rose to control values (Fig 4)

In the anorexic patients who exercised at 12 C E was suppressed completely $\bar{T}_{sk}$ was similar to the controls value but again the temperature of the hand decreased to approximately 16 C

DISCUSSION

In a moderate environment AN patients appear to regulate their body (core) temperature in a way similar to that found in extremely sedentary and unacclimatized subjects (23) At rest the anorexia patients have low T_{re} (Table II) and body heat content this gives rise to a greater capacity for S and delayed increase of T_{re} observed at the onset of work During exercise at a dry bulb temperature of 24 C they have a higher T_{re} for a given M than control subjects but E for a given H was within the expected range previously found for normal subjects (Table II and Fig 3) Passively heating the patients before exercise raised resting T_{re} and increased E but it did not effect the rate of change in T_{re} during exercise nor the final plateau value of T_{re} at the end of work In relation to relative work load the T_{re} at the end of exercise lie within the lower

range of values previously found for normal subjects (7 18)

A slightly reduced T_{re} for a given % V_{O_2} max might suggest that the loss of thermal insulation in the form of body fat may have a small effect on thermoregulation in AN However some caution is necessary in interpreting the results We have alluded to the difficulties of measuring V_{O_2} max directly in AN patients (8) In normal subjects a slight bias in V_{O_2} max may be less critical but in patients who have an extremely low V_{O_2} as in AN it has a decisive effect For example an underestimation of (say) 200 ml/min $^{-1}$ in a patient with V_{O_2} max of 1 5 l/min $^{-1}$ will result in an error of at least 10% in the calculation of relative work load This factor could easily account for the small differences in and the larger intersubject variability of T_{re} for a given V_{O_2} max in 7 of the 10 patients studied compared with normal subjects (Fig 2) but it could not explain the data on the remaining three patients exercising at 24 C nor the results on the patient at 12 C Undoubtedly a severe loss of body fat in AN will facilitate the convective transfer of heat from the core to the skin surface and it was observed that a greater proportion of H of AN patients even in a moderate environment is dissipated by convection and radiation ($C+R$) However without knowledge of minimal conductance values in air for these anorexic patients it is impossible to decide whether part of this heat loss by $C+R$ is obligatory due to their reduced thermal insulation or a result of active physiological regulation A reduction in the amount of heat to be actively regulated would be expected to result in a lower T_{re} for a given M expressed as % V_{O_2} max and ultimately to have a decisive effect on thermoregulatoin (16)

In cool conditions (12 C) the patient despite the increase in M during exercise by shivering (Table II) was barely able to maintain his body temperature above resting conditions The response by shivering in cold environment is contrary to reports by others (20) Sweating was suppressed and the heat loss from the body was solely due to respiration and $C+R$ In the control subjects T_{re} rose to the same plateau value as observed at 24 C though E was reduced and $C+R$ increased This suggests that the loss of body fat in anorexia may effect a reduction in the prescriptive (12 13) range of ambient temperatures through which body temperature can be maintained and physiologically regulated This is clearly an area for further research

In warm conditions the thermoregulatory problems for the anorexic patient are the reverse of those encountered in a cool environment for though the loss of fat facilitates the convective transfer of heat from core to skin the raised ambient temperature reduces $C+R$ losses to the environment In order to maintain thermal equilibrium the anorexic patient must sweat They are clearly capable of doing this (Table II and Fig 3) at 32 C E increased by 64% during exercise in 2 patients over the value observed at 24 C However the maximal sweating capacity of the anorexic patient seems to be low For example during exercise at 32 C one of the two patients studied showed signs of heat exhaustion and developed a pyrexia rash and the experiment had to be terminated before the 60th min at a T_{re} of 38 2°C (pat 2 Fig 1) None of the control subjects showed such signs when exercised under similar conditions

Thus anorexic patients appear to differ from normal subjects in their low sweating capacity and in the proportions of heat lost from the skin by evaporative and non-evaporative channels In a moderate environment they behave from the thermoregulatory point of view in a way qualitatively similar to normal subjects exercising in a cool environment This they appear to achieve to some degree by selective control of vasoconstrictor tone particularly in the extremities for although the anorexic patient maintains a slightly higher T_{sk} than controls (Table II) the T_{sk} of their limbs is extremely low (Fig 4) Presumably in an attempt to conserve and control the amount of heat lost from the body surface by $C+R$ the patient limits the size of his core by maintaining active vasoconstrictor tone in his extremities (2 19) Under these conditions it has been shown (2 19) that heat can be directly exchanged by a counter current mechanism since blood is diverted from the superficial to the deep veins which lie in close proximity to arteries which carry the heat from the core to the periphery In our view the present data provide no support for the theories of Wakeling and Russell (21) and Mecklenburg et al (14) that AN produces a primary (hypothalamic) dysfunction of the temperature regulatory system T_{re} appears to reach a plateau value independent of the initial change in H at the onset of exercise and the relative proportions of heat lost by E and $C+R$ The physiological effect of AN may be to narrow the prescriptive limits of temperature regulation the clinical consequences

of which would be the risk of accidental hypothermia if the patient is exposed to a cool or cold environment without adequate protection

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REFERENCES

1 Åstrand P O Experimental studies of physical working capacity in relation to sex and age Munksgaard Copenhagen 1952
2 Bazett H C Love L Newton M Eisenberg L Day R & Forster R Temperature changes in blood flowing in arteries and veins in man J Appl Physiol 1 3 1948
3 Beumont P J V Anorexia nervosa S Afr Med J 44 911 1970
4 Bittel J & Henane R Comparison of thermal exchanges in man and women under neutral and hot conditions J Physiol 250 475 1975
5 Dally P Anorexia nervosa Heinemann Medical Books London 1969
6 Davies C T M Limitations to the prediction of maximum oxygen intake from cardiac frequency measurements J Appl Physiol 24 700 1968
7 Davies C T M Brotherhood J & Zeidifard E Temperature regulation during severe exercise with some observations on the effects of skin wetting J Appl Physiol 41 772 1976
8 Fohlin L Bjarke B Davies C T M Freyschuss L & Thoren C Function and dimensions of circulatory system in anorexia nervosa Acta Paediatr Scand 67 11 1978
9 Fohlin L Body composition cardiovascular and renal function in adolescent patients with anorexia nervosa Acta Paediatr Scand (Suppl) 268 1977
10 Gleeson C & Moore R E Investigation of heat control in anorexia nervosa Ir J Med Sci 143 337 1974
11 Hardy J E & Dubois E F The technic of measuring rediation and convection J Nutr 15 461 1938
12 Lind A R A physiological criterion for setting thermal environmental limits for everyday work J Appl Physiol 18 51 1963
13 — Tolerable limits for prolonged and intermittent exposure to heat In Temperature Its measurement and control in science and industry (ed J D Hardy) 3 337 Reinhold New York 1963
14 Mecklenburg R S Loriaux D L Thompson R H Andersen A E & Lipsett M B Hypothalamic dysfunction in patients with anorexia nervosa Medicine 53 147 1974
15 Nielsen B Thermoregulation in rest and exercise Acta Physiol Scand (Suppl) 323 1969
16 Nielsen B & Davies C T M Temperature regulation during exercise in water and air Acta Physiol Scand 98 500 1976
17 Pařízková J Total body fat and skinfold thickness in children Metabolism 10 694 1964
18 Saltin B & Hermansen L Esophageal rectal and muscle temperature during exercise J Appl Physiol 21 1757 1966
19 Schmidth Nielsen K Heat conversation in counter current system In Temperature Its measurement and control in science and industry (ed J D Hardy) 3 143 Reinhold New York 1963
20 Vigersky R A & Loriaux D L Anorexia nervosa as a model of hypothalamic dysfunction in anorexia nervosa Raven Press New York 1977
21 Wakeling A & Russell G F M Disturbances in regulation of body temperature in anorexia nervosa Psychol Med 1 30 1970
22 Warren M P & Wiele R L V Clinical and metabolic features of anorexia nervosa Am J Obstet Gynecol 117 435 1973
23 Wyndham C H Strydom N B Munro A Macpherson R K Metz B Scheff G & Schieber J Heat reactions of Caucasians in temperature in hot dry and in hot humid climates J Appl Physiol 19 607 1964

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Cytologic Diagnosis of Thyrotoxicosis

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ABSTRACT In specimens obtained by fine needle thyroid puncture it is possible to define cytologic criteria of toxic goiter This cytologic picture is compound but the authors describe *marginal vacuolization* and *nuclear ring patterns* in follicular wall fragments as fairly specific signs of thyroid hyperfunction Thyroid puncture is hardly a measure of choice in the routine clinical evaluation of hyperthyrosis but some case histories demonstrate that due attention to the cytologic picture of toxic goiter is profitable also when the puncture was made with quite other indications

Key words thyrotoxicosis cytologic diagnosis thyroid puncture

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Fine needle thyroid puncture is a valuable source of information regarding thyroiditis thyroid cysts and tumors it may seem out of place to use it for the diagnosis of a functional disturbance such as thyrotoxicosis There is however a distinctive cytologic picture also of toxic goiter which may help to reveal the presence of overlooked thyrotoxicosis and hot nodules Cytologic criteria of toxic goiter will in this paper be presented together with some casuistic illustrations of their usefulness in cases of difficult diagnosis

Attempts to define such criteria have been made for a long time The large granulated cells believed by Tempka et al (8) to be hyperactive glandular cells are now recognized as follicular macrophages common in most multinodular goiters The paravacuolar granules observed by Soderstrom (6) in follicular cells from toxic goiters are probably lysosomal structures not consistently correlated to thyrotoxicosis Myren and Sivertssen (1) noted increased nuclear size and Nilsson (2) a discontinuous variation in nuclear size (Jakobj phenomenon) in toxic goiters but these features have unfortunately a low degree of specificity More specific is a tendency of nuclei to form ring patterns in follicular fragments from toxic goiters (Fig 1) an expression in terms of aspirate cytology of the high epithelium pattern in histology (7) A fairly specific feature is the curious marginal vacuolization of follicular epithelium reported by Nilsson (3) numerous large vacuoles especially at the margins of follicular wall fragments often staining pale pink with May-Grunwald Giemsa (Fig 2) They may *ad interim* be regarded as *significant aspiration* artefacts as they are not seen in histology It may be added that increased numbers of lymphocytes and even interstitial C cells have also been observed in aspirates from toxic goiters (4 5) Though theoretically interesting these findings do not rank high as diagnostic arguments for toxic goiter

From our present experience cytologic criteria suggesting toxic goiter may be ranked as follows 1) *Marginal vacuolization* of follicular epithelium cells 2) *Nuclear ring pattern* in follicular wall fragments 3) *Some other findings* (large nuclei Jacobj type anisocaryosis and excess admixture of lymphocytes) which are non specific but nevertheless contribute to the picture of toxic goiter in terms of cell ecology

MATERIAL AND METHODS

The authors include thyroid puncture in the routine examination of all palpable goiters met with in apparently *euthyroid* patients Using hypodermic needles and disposable 10 ml syringes we puncture nodule(s) and internodular tissue separately at least two punctures are thus usually performed at the same sitting The material obtained is prepared as air-dried smears and stained with May Grunwald-Giemsa as ordinary blood smears

We usually omit puncture in the presence of overt hyperthyrosis there is a slightly increased risk of hematoma in the hyperemic toxic gland and when this diagnosis is already in focus we do not expect important additional information from cytology

Observations

In spite of the policy mentioned we now and then with a toxic goiter cytology also in this material

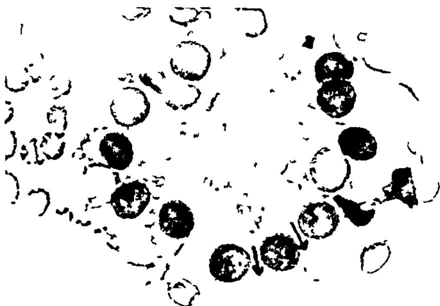


Fig 1 Foll cular wall frag ment in a p rate from tox c go ter H gh foll cular ep the l um result ng in nuclear ring pattern n the smear Rather few marginal vac uoles (*arrows*) (Air dried smear May Grunwald G emsa ×9 0)

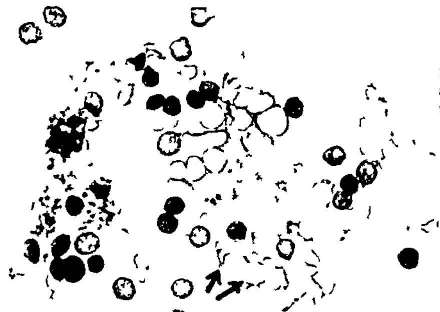
consecut ve series of 100 thyro d punctures rev ewed in retrospect a tox c cytologic p cture had thus been re corded n 8 cases In 4 of these cases a hyperthyrosis proved really to be present though overlooked or perhaps cl n cally latent at the pat ent s f rst visit The follow ng case histories illustrate the d agnostic contr butions to be expected from cytology

CASE HISTORIES

Case 1

An ass stant nurse aged 63 s nce her 20s aware of a small nodular go ter For about the last 5 years adult type dia betes treated w th d et and an athelecta s in her left lung of obscure etiology F rst seen by one of us n Sept 1977 compla n ng of general lass tude gastr c pa ns anorex a loss of we ght and some breathlessness

At the first exam nation the small go ter was noted but there was no impression of thyrotox cos s and the pat ent d d not allow puncture Gastric troubles a sl ghtly en larged l ver and a somewhat elevated ESR d rected atten tion to the al mentary tract and a search for mal gnancy was started Noth ng was found but her general cond t on deteriorated w th d arrhea cont nuous loss of we ght and personality changes observed by her daughter In Dec 1977 she allowed thyro d puncture and 3 a p rates all



F g 2 Foll cular wall frag ment n asp rate from tox c go ter Numerous marg nal vacuoles (*arrows*) (A r dried smear May Grunwald G emsa ×368)

revealed the cytologic picture of toxic goiter (marginal vacuolization and nuclear ring pattern) This directed attention to hyperthyroid features in her clinical status (deep tremor warm and moist skin brisk tendon reflexes) and her serum hormone levels proved impressively elevated (serum thyroxine 250 nmol/l free thyroxine 212 pmol/l serum triiodothyronine 6 0 nmol/l free triiodothyronine 61 pmol/l)

After ^{131}I treatment the patient soon became euthyroid and made a very satisfactory recovery

Case 2

A washerwoman aged 62 who had since her first pregnancy at the age of 28 suffered from obesity (body weight around 95 kg) A mild diabetes treated with diet had been present for three years For many years she had consulted psychiatrists for anxiousness and had consumed a large amount of anxiolytic drugs

At a routine control she complained of increased nervousness and a globus feeling and a small multinodular goiter was detected and punctured There seemed to be nothing in the clinical picture to suggest hyperthyrosis but the aspirates revealed the picture of toxic goiter with large clear nuclei and numerous marginal vacuoles A reappraisal of her clinical picture disclosed that she had experienced a specific type of unrest diminished heat tolerance and loss of weight during the last year The patient had a warm and moist skin but no tachycardia and no endocrine eye symptoms Serum thyroxine was 160 nmol/l serum triiodothyronine 2 5 nmol/l and T_3 resin uptake index 125% It was concluded that the patient had a mild hyperthyrosis ^{131}I treatment greatly improved her general condition and especially her nervous symptoms

Case 3

A married district nurse aged 35 She had not herself been aware of a small asymmetric but not distinctly nodular goiter detected in May 1975 by the district medical officer who suspected a thyrotoxicosis When one of the authors saw the patient 2 months later she was clinically euthyroid and had serum thyroxine and triiodothyronine resin uptake values well within the normal range (75 nmol/l and 92% respectively)

Routine thyroid puncture disclosed however in two samples from the enlarged right lobe a toxic goiter cytology with marginal vacuolization and typical nuclear rings A sample from the left lobe showed normal epithelium cyst macrophages and some microfollicles—thus the usual finding in non toxic nodular goiter A technetate scintigram confirmed the presence of a right sided (subtoxic) hot nodule with suppression of the technetate uptake in the rest of the gland

Somewhat later this patient developed an overt hyperthyrosis and the nodule had to be surgically removed

DISCUSSION

Cases 1 and 2 represent the well known experience that hyperthyrosis is often overlooked in elderly patients in these cases it was not considered until the typical cytologic picture suggested its presence Case 3 illustrates the self-evident fact that the cytologic picture in a single aspirate reflects nothing more than the conditions prevailing in the part of the thyroid punctured in this case a hot adenoma Such a regional hyperfunction is probably a common phenomenon also in cases without clinical hyperthyrosis

This communication is not intended to advocate thyroid puncture as a routine test for the diagnosis of hyperthyrosis when this problem is in focus With the increasing use of thyroid puncture for other indications however the cytologic picture of toxic goiter will often turn up as an accidental finding If it is recognized and reported it will as often become an important contribution to the clinical diagnosis

REFERENCES

1 Myren J & Sivertssen E Thin needle biopsy of the thyroid gland in the diagnosis of thyrotoxicosis Acta Endocrinol 39 431 1962
2 Nilsson G Nuclear size classes in fine needle aspirates from toxic goiters Acta Endocrinol 70 273 1972
3 — Marginal vacuoles in fine needle aspirates from toxic goiters Acta Pathol Microbiol Scand 80 289 1972
4 — Cytology of toxic goiters as studied in fine needle aspirates Studentlitteratur Lund 1972
5 — C-cells in non malignant human goiters studied in fine needle aspiration biopsy specimens Acta Med Scand 191 244 1972
6 Soderstrom N Puncture of goiters for aspiration biopsy Acta Med Scand 144 237 1952
7 — Fine needle aspiration biopsy Almqvist & Wiksell Stockholm 1966
8 Tempka T Aleksandrowicz J & Till M Le thyroidogramme Sang 19 336 1948

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The Influence of Endogenous Cortisol on the Peripheral Conversion of Thyroxine in Patients with Acute Myocardial Infarction

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ABSTRACT A study was performed to elucidate whether endogenous cortisol, as previously suggested could be responsible for the decreased T_3 levels seen in euthyroid patients with acute myocardial infarction Levels of these hormones as well as levels of T_4 and reverse T_3 were monitored in 31 consecutive patients admitted to the Coronary Care Unit with symptoms of precordial pain or with acute arrhythmias Sixteen of the patients had proven myocardial infarction the remaining 15 were used as a control group The results demonstrated that a reduction of T_3 levels was seen in the infarction group without evidence of a statistically significant difference between the daily mean cortisol levels No significant difference could be observed in T_4 or reverse T_3 levels in the two groups or in T_3 levels in the control group It is concluded that the decrease in T_3 levels is not a consequence of the increased levels of endogenous cortisol

Key words thyroxine triiodothyronine reverse triiodothyronine acute myocardial infarction thyroxine conversion cortisol

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It is now generally recognized that reduced serum triiodothyronine (T_3) levels can often be seen in euthyroid patients with various acute and chronic non thyroidal illnesses and after starvation or malnutrition (3 6 8 15) Some investigators have also noted a simultaneous increase in the levels of 3 3 5 triiodothyronine (reverse T_3) in these conditions (4 7 16) The mechanism responsible for these decreased levels is not known An altered peripheral metabolism of thyroxine (T_4) has been held to be the most likely explanation Support for this conclusion is that the levels of TSH and T_4 were normal

An increased secretion of corticosteroids has been one of the factors discussed in connection with the mechanism underlying the altered peripheral conversion of T_4 (5 10 11 17) This has been explained on the basis of the demonstration of the inhibitory effect of corticosteroids on the conversion of T_4 to T_3 and on the possibility of increased cortisol secretion in these conditions

Acute myocardial infarction (AMI) is one condition in which an increased secretion of corticosteroids is observed (2 9 14) and in which an altered peripheral conversion of T_4 to T_3 and reverse T_3 has been found (12 18)

The aim of the present study was to elucidate whether endogenous cortisol could be responsible for the decreased T_3 levels seen in patients with AMI

PATIENTS AND METHODS

Thirty-one consecutive patients 26 men and 5 women aged 65 years or younger admitted to our Coronary Care Unit were included in the study The mean age (±S D) of the men was 55 1±11 5 years (range 19–65) and of the women 56 8±5 4 years (range 52–63) All patients were admitted with symptoms of precordial pain or with acute arrhythmias All patients were treated initially with bed rest and all received a regular hospital diet Blood samples were taken ECGs recorded and other relevant tests performed according to standard procedures The first test samples were taken within the first hour after admission to the ward while all further tests were made each morning before breakfast Body temperature was recorded 1–1½ hours before blood sampling on all days except the day of admission when it was measured only sporadically All drugs were recorded in the case reports

T_4 and T_3 levels were determined by a radioimmunoassay technique recently described (13) The normal level

Abbreviations T_3=3,5 3 triiodothyronine reverse T_3= 3 3 ,5 triiodothyronine T_4=thyroxine AMI=acute myocardial infarction

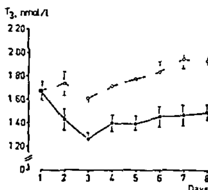


Fig 1 Serum T_3 concentrations (mean±S E M) in the infarction group (●) and the controls (O) Infarction day=day 1

(mean±S D) for T_4 is 89±17 nmol/l and for T_3 1 77±0 34 nmol/l All samples were frozen at −20°C and analyzed simultaneously

Reverse T_3 was determined by a commercial kit (Hypolab Coinsins Switzerland) The normal level (mean±S D) is 0 34±0 20 nmol/ All samples were frozen at −20°C and analyzed simultaneously

Cortisol was determined by a commercially available radioimmunoassay kit using the double antibody separation technique (Diagnostic Products Los Angeles California USA) The normal level for circulating cortisol at 8 00 a m is 150–600 nmol/l

The statistical evaluation was performed by Dr Ekblom Department of Mathematical Statistics University of Stockholm

RESULTS

ixteen of the 31 patients demonstrated features of proven myocardial infarction with typical ECG changes and enzyme patterns The diagnoses of the remaining 15 patients included angina pectoris in 7 patients acute perimyocarditis in 2 cardiac arrhythmias in 4 congestive heart failure in one and a myocardial infarction 2 weeks prior to admission in one patient These 15 patients functioned as controls in the study Fifteen of the patients with myocardial infarction were men mean age 55 1 years (range 36–65) Eleven in the control group were men mean age 55 7 years (range 19–65)

T_4 T_3 and reverse T_3 No significant difference could be observed between levels of T_4 and reverse T_3 either between the two groups studied or in the daily determinations of these parameters throughout the study On the other hand a slight elevation in reverse T_3 levels could be seen in the infarction group between days 2 and 5 However as shown in Fig 1 a significant decrease in mean T_3 levels was found between the first day and the following four days in the infarction group The serum T_3 levels in this group were reduced from an initial mean of 1 67 (±0 08) nmol/l to a minimum of 1 26 (±0 05) nmol/l on the third day This difference was highly significant ($p<0\ 001$) No significant decrease in mean T_3 levels was found throughout the study in the control group A significant difference was found between the mean values of T_3 levels in the two groups subsequent to the first day

Cortisol The results are shown in Fig 2 No significant difference was found in mean cortisol levels between the first day and the following five days in the infarction group A significant decrease ($p<0\ 05$) was found on the 7th and 8th days Throughout the study no significant difference was found in mean cortisol levels within the control group However a significant difference was found between the mean cortisol levels in the two groups on days 2 3 and 5

Drugs The evaluation of the drugs given to the patients in the two groups revealed no major differences except that 16 patients in the infarction group received a mild laxative compared to 9 in the control group and 11 in the infarction group against 6 in the non infarction group received a small amount (max 15 mg a day) of diazepam

DISCUSSION

Previous reports have suggested that cortisol is involved in the peripheral metabolism of T_4 in patients with myocardial infarction (12 18) This suggestion was based upon the observation that glucocorticoids can alter the peripheral metabolism of T_4 in the same way as seen in various non

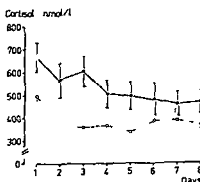


Fig 2 Serum cortisol concentrations (mean±S E M) in the infarction group (●) and the controls (O) Infarction day=day 1

thyroidal illnesses with increased production of glucocorticoids However the dose of glucocorticoids used in the studies was unphysiologically high (5 10 11 17)

The present study confirms previous observations of a significant reduction of serum T_3 levels in patients with AMI (12 18) The results also show that no significant T_3 decrease could be demonstrated in the control group and that the mean serum cortisol levels in the infarction group were significantly higher than in the control group Thus increased cortisol levels could be considered responsible for the decreased T_3 levels

However in the infarction group the reduction of T_3 levels was seen without a statistically significant difference between the daily mean cortisol levels *This observation suggests that the increase in* serum cortisol has no significant effect on T_3 levels Furthermore no significant correlation was found between each cortisol value and the corresponding T_3 value determined in the same serum specimen The difference in mean cortisol levels between the two groups was smaller than the differences seen during the normal diurnal cortisol rhythm furthermore this rhythm does not affect the T_3 levels since no diurnal rhythm has been observed for T_3 (1)

Thus it can be concluded from the present results that the decrease in T_3 levels is most likely *not* caused by increased levels of endogenous cortisol

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REFERENCES

1 Azukizawa M Pekary A E Hersman J M & Parker D C Plasma thyrotropin thyroxine and triiodothyronine relationships in man J Clin Endocrinol Metab 43 533 1976

2 Bailey R R & Abernethy M H Adrenocortical response to the stress of an acute myocardial infarction Lancet 1 970 1967

3 Bermudez F Suchs M J & Oppenheimer J H High incidence of decreased serum triiodothyronine concentration in patients with non thyroidal disease J Clin Endocrinol Metab 41 27 1975

4 Burger P Nicod T Suter P Vallatton M D Vagenakis A & Braverman L Reduced active thyroid hormone levels in acute illness Lancet 2 653 1976

5 Burr W A Ramsden D B Griffiths R S & Black E G Effect of a single dose of dexamethasone on serum concentrations of thyroid hormones Lancet 2 58 1976

6 Carter J N Eastman C J Coccocan J M & Lazarus L Effect of severe chronic illness on thyroid function Lancet 2 971 1974

7 Chopra I J Chopra U Smith S R Reza M & Salomon D H Reciprocal changes in serum concentrations of 3 3 5 triiodothyronine (reverse T_3) and 3 5 3 triiodothyronine (T_3) in systemic illness J Clin Endocrinol Metab 41 1043 1975

8 Chopra I J & Smith S R Circulating thyroid hormones and thyrotropin in adult patients with protein-calorie malnutrition J Clin Endocrinol Metab 40 221 1975

9 Chopra M P Thadani U Aber P Portal R W & Parkes J Plasma cortisol urinary 17 hydroxycorticoids and urinary vanilyl mandelic acid after acute myocardial infarction Br Heart J 34 992 1972

10 Chopra I J Williams D E Orngazzi J & Solomon D H Opposite effects of dexamethasone on serum concentrations of 3 3 ,5 triiodothyronine (reverse T_3) and 3 5 3 triiodothyronine (T_3) J Clin Endocrinol Metab 41 911 1975

11 Degroot L J & Hoye K Dexamethasone suppression of serum T_3 and T_4 J Clin Endocrinol Metab 42 976 1976

12 Kaplan M Schimmel M & Utiger R D Changes in serum 3 3' 5 triiodothyronine concentrations with altered thyroid hormone secretion and metabolism J Clin Endocrinol Metab 45 447 1977

13 Ljunggren J G Persson B & Tryselius M Rapid simultaneous radioimmunoassay for the measurement of triiodothyronine and thyroxine on unextracted human serum Acta Endocrinol (Kbh) 84 487 1976

14 Logan R W & Murdoch W R Blood levels of hydrocortisone transaminases and cholesterol after myocardial infarction Lancet 2 521 1966

15 Portnay G I O Brian J T Burk J Vagenakis A G Azizi F Arky R A Ingbar S H & Braverman L E The effects of starvation on the *concentration and binding of thyroxine and triiodo*thyronine in serum and on the response to TRH J Clin Endocrinol Metab 39 191 1974

16 Vagenakis A G Burger A Portnay G I Rudolph M O Brian J T Azizi F Arky R A Nicod P Ingbar S H & Braverman L E Diversion of peripheral thyroxine metabolism from activating to inactivating pathways during complete fasting J Clin Endocrinol Metab 41 191 1975

17 Westgren U Ahren B Burger A Ingemansson S & Melander A Effects of dexamethasone desoxycorticosterone and ACTH on serum concentrations of thyroxine 3,5 3 triiodothyronine and 3 3 5 triiodothyronine Acta Med Scand 202 89 1977

18 Westgren U Burger A Levin K Melander A Nilsson G & Pettersson U Divergent changes of serum 3,5 3 triiodothyronine and 3 3 5 triiodothyronine in patients with acute myocardial infarction Acta Med Scand 201 69 1977

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Occurrence of Acute Myocardial Infarction in Stockholm Studied by Two Different Methods

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ABSTRACT Two studies on the occurrence of acute myocardial infarction (AMI) in Stockholm have been made In one the study population consisted of patients who attended a health control station, cases of AMI were detected retrospectively by a mail questionnaire and by the official cause-of-death register In the other study the population comprised the total population of Stockholm county cases of AMI were detected from routine information on hospital care, and from the official cause-of-death register The aims of the present study were to compare the two methods for detection of AMI cases and to compare the AMI morbidity of the two populations The mail questionnaire method missed a small number of cases but on the other hand, the register method included some cases in which the diagnostic criteria were not fulfilled The AMI morbidity in the two populations did not differ significantly

Key words epidemiology myocardial infarction register data

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Studies on the occurrence of acute myocardial infarction (AMI) have employed various methods for detecting events of AMI Concerning the Stockholm area there exist two studies which have used different methods for this purpose One of them the Stockholm Prospective Study (SPS) was based on a mail questionnaire and the other the Stockholm County Study (SCS) on routinely collected computer data One aim of the present study was to compare the two methods

There are several epidemiological studies of AMI in which the population under study does not comprise a random sample from the general population of an area but e g the members of a labour union the employees in an industry or the individuals insured in an insurance-company It is quite possible that such populations are biased in some respect They may for instance consist of people with better health than the general population in the area The presence of bias could of course influence the incidence rates However it could also influence the estimated relationships between risk indicators and the disease The latter fact is clearly demonstrated by Rothman (11)

Since the study population in the SPS comprised people examined at a health control center a bias of the type discussed above could not be excluded Therefore another aim of the present study was to compare the AMI morbidity in the SPS population with that of the general population in the area as measured in the SCS

STUDY POPULATION

Stockholm Prospective Study

In 1961–62 the concentrations of plasma triglycerides cholesterol Hb and ESR were analyzed in 6 464 men and women who attended a health-control station In addition information about their weight height blood pressure physical activities and smoking habits were recorded (5) The health station is owned by a number of companies and the study was made as a part of a yearly check up offered to employees

In 1971 and 1976 follow up studies were carried out (4) All deceased individuals were traced by comparing the files of the SPS with the official mortality files of the National Central Bureau of Statistics (NCBS) For all deceased persons death certificates and autopsy reports were checked In this way fatal cases of AMI were recorded A simple questionnaire was mailed to the surviv

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Abbreviations AMI=acute myocardial infarction SCS=Stockholm County Study SPS=Stockholm Prospective Study NCBS=National Central Bureau of Statistics SMR=standardized morbidity ratio

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ing population The subjects were asked if they had ex perienced and AMI since 1961 and if so where and when

The hospital records of those who reported an AMI were analyzed and in a living patient the diagnosis was ac cepted if at least two of the following criteria were ful filled *a*) Clinical history (typical clinical history with acute severe retrosternal pain with or without frank pulmonary edema or shock) *b*) Positive ECG findings (appearance of a Q wave or S T elevations followed by subsequent T inversions in a localized area) *c*) Positive serum enzyme pattern (typical enzyme pattern with an increase in serum GOT GPT or LDH followed by normalization of the values in a characteristic time course) Myocardial infarction in a dead hospitalized patient was diagnosed from the same criteria and from autopsy findings

In this study only one event of AMI for each subject was recorded The total number of detected fatal and non fatal cases of AMI during the period 1962–75 was 199

Stockholm County Study

The SCS used hospital stays with a diagnosis of AMI recorded on discharge from hospital This was done by means of a computerized in patient care register kept by the Stockholm County Council In addition data were obtained from the cause-of-death register kept by the NCBS Hospital stays and deaths were brought together to give cases of AMI which had occurred in 1973 The number of person years in the population of Stockholm county distributed by sex and age was calculated by use of a population register which is kept by the Stockholm County Council Age and sex specific incidence rates were then calculated by dividing the number of AMI cases by the number of person years in the corresponding age and sex group In SCS the number of recorded cases of AMI in 1973 was 4073 The population base was 1 5 mill (1)

METHODS

In order to compare the two methods for detection of AMI a record linkage was performed The ten-digit civil registration number of each subject was used for identification For each subject in SPS a check was made of whether an AMI had been recorded in 1973 according to the SCS method and if so whether the case had also been recorded by the SPS method Similarly for all events of AMI recorded by the SPS method a check was made of whether they had also been recorded in the SCS material

In order to compare the AMI morbidity in the SPS population with that in the SCS population the observed number of cases of AMI in the SPS population (*O*) was compared with an expected number (*E*) The expected number of cases of AMI was calculated as

$$E=\Sigma N_i I_i \quad i=1\ 2$$

where N_i=the number of person years in the *i*th 5 year age category in the SPS population and I_i=the incidence rate in the same category as calculated in the SCS

As a measure of the discrepancy in morbidity in AMI between the two populations the standardized morbidity ratio (SMR) was used This was defined as

$$SMR=O/E\times 100$$

Thus confounding by age was avoided by the use of indirect standardization (3)

The hypothesis that SMR=100, i e that the morbidity in the two populations was the same was tested by the test variable

$$\chi^2=(O-E)^2/E$$

which was supposed to be distributed according to the χ^2 distribution with 1 d f under the null hypothesis Test based 95% confidence limits were assessed as

$$SMR\ (1\pm 1\ 96/\chi)$$

according to a method suggested by Miettinen (9) Exact confidence limits for a proportion were obtained in ac cordance with Clopper and Pearson (6)

RESULTS

In the 1976 follow up of the SPS population 24 subjects were found to have had AMI in 1973 When the SPS population was located in the SCS material however 34 subjects with AMI were identified Of these 21 subjects appeared in both studies (Table I)

The 13 subjects with AMI in 1973 according to the SCS study who were not identified in the SPS study were further analyzed Reasons why 13 cases detected by the SCS were not noted in the SPS are 6 had also had an AMI prior to 1973 which was the one recorded in the SPS while the AMI in 1973 was not noted Further 5 individuals had had an AMI according to SCS and had given a positive answer to the SPS questionnaire However on examination of the records they had not fulfilled the diagnostic criteria used in the SPS Two cases of AMI were detected by the SCS method but not by the SPS questionnaire These subjects had been treated in hospital for AMI in 1973 but had given a negative answer to the SPS questionnaire Thus out of 29

Table I *Comparison of events of AMI in 1973 detected by the two studies*

AMI according to SPS	AMI according to SCS Yes	No	Total
Yes	21	3	24
No	13	–	–
Total	34	–	–

Table II *Observed number of AMIs during 1973 in the SPS as detected by the SCS and expected number of AMIs in the SPS as calculated from SCS incidence rates for the same period*

	Males	Females	Total
Observed	28	6	34
Expected	30 9	6 3	37 1
SMR	91	–	92
95% confidence interval	63–131	–	65–128
p	0 61	–	0 61

cases that should have been recorded 7% (2 cases) were missed The corresponding 95% confidence interval was 1–24%

Three individuals with AMI were identified by the SPS method but not by the SCS Two of these subjects lived outside Stockholm county and were therefore not included in the study population of the SCS One person had had an AMI in 1973 but was treated in a hospital outside the county and had accordingly not been recorded in the hospital care register kept by the Stockholm County Council

The observed AMI cases in the SPS population in 1973 as detected by the SCS method are compared in Table II with the expected number of such cases For both sexes the SMR was 92 with 95% confidence limits of 65 and 128 The significance test gave p=0 61

Using the incidence rates for AMI for 1973 in the SCS material and the total number of person years lived in the SPS population in 1962–75 the expected number of AMIs during this whole period was calculated This was 389 while the observed number was 199 and the SMR 51 A major explanation for the large difference was the fact that only one event of AMI had been recorded per subject in the SPS Henning and Lundman (8) found that 37% of all cases of AMI were reinfarctions Utilizing this information a corrected SMR was calculated This was 81 which still differed significantly from 100 (p=0 003)

DISCUSSION

The comparison of the two methods for AMI detection showed that the SPS questionnaire method missed 7% (2/28) of the subjects with the disease in 1973 according to the SCS register method The precision of this estimate is low as shown by the confidence interval However the result is in agreement with findings from previous studies on underreporting of health events (10) On the other hand none out of 21 cases that should have been detected by the SCS method according to the SPS was missed One subject in the SCS population had had an infarction which was not detected because he had been treated in hospital outside the county

The above findings show that a medical information system of the kind used in the SCS is a valuable tool in epidemiological studies In the use of register data the hospital records can be checked to avoid false positive diagnoses of AMI However it must be kept in mind that the false negatives cannot be corrected for but neither can this be done when the mail questionnaire method is used

A separate study has shown that the frequency of such false positives is about 6% (2) For this the criteria in a Swedish co-operative CCU study were used (8) These were as follows (*a*) Central chest pain pulmonary oedema syncope or shock (*b*) Appearance of a pathological Q wave and/or appearance or disappearance of a localized ST elevation followed by a T inversion in two or more of the 12 leads (*c*) Two SGOT values of 40 U or more with a maximum about 24 hours after onset of symptoms in combination with a SGPT maximum after about 36 hours and lower than the SGOT maximum (*d*) Findings at autopsy of myocardial necrosis of an age corresponding to the onset of symptoms Either two of criteria *a* *b* and *c* or criterion *d* should be fulfilled

Without correction for either the false positives or the false negatives the incidence rates established in the SCS were very similar to those found in an extensive WHO cooperative study from Gothenburg (1 7)

The comparison of the morbidity for 1973 between the SPS and SCS showed no differences as far as the incidence of AMI was concerned This would indicate that the SPS population was not biased in the sense that it had a morbidity different from that of the general population of Stockholm county On the other hand the number of cases was small resulting in a wide confidence interval for the SMR

Further the expected number of AMIs during the period 1962–75 was compared with the observed number In this comparison unfortunately the observed number had to be assessed by the SPS

method but the expected by the SCS method This comparison however showed highly significant differences between the two studies The observed number was lower than the expected The main reason for this discrepancy was the fact that in SPS only one event of AMI was noted per subject When the incidence rates in the SCS study were corrected for reinfarctions the SMR for both sexes together still differed significantly from 100 ($p=0\ 003$)

It was seen previously that the SPS mail questionnaire method missed some proportion of the cases of AMI noted by SCS This could explain part of the discrepancy If the observed number in 1962–75 is corrected for this by increasing by 10% the resulting SMR would be 89 which would not *differ significantly from 100* ($p=0\ 09$) *Thus even if* the precision of the estimated proportion is low it is likely that the rest of the discrepancy could be explained in this way

In summary it was concluded that the morbidity of the SPS population was similar to that of the SCS population It was also found that the SPS mail questionnaire method missed a smaller number of AMI cases than SCS

REFERENCES

1 Ahlbom A Acute myocardial infarction in Stockholm —A medical information system as an epidemiological tool Am J Epidemiol In press 1978
2 Ahlbom A & Nordlander R Application of diagnostic criteria in the diagnosis of myocardial infarction Scand J Soc Med In press 1978
3 Armitage P Statistical methods in medical research Further analysis of qualitative data Blackwell Scientific Publications Oxford London Edinburgh and Melbourne 1971
4 Bottiger L E & Carlson L A The Stockholm Prospective Study 2 New events of coronary heart disease in men in relation to findings at initial examination 9 year follow up Skandia International Symposia Early phases of coronary heart disease p 158 Nordiska Bokhandelns Forlag Stockholm 1973
5 Carlson L A & Lindstedt S The Stockholm Prospective Study 1 The initial values for plasma lipids Laromedelsforlagen Svenska Bokforlaget Stockholm 1968
6 Clopper C J & Pearson E S The use of confidence or fiducial limits illustrated in the case of the binomial Biometrika 26 404 1939
7 Elmfeldt D Wilhelmsen, L Tibblin G Vedin J A Wilhelmsson C E & Bengtsson C Registration of myocardial infarction in the city of Goteborg Sweden J Chronic Dis 28 173 1975
8 Henning R & Lundman T Swedish co-operative CCU study A study of 2008 patients with acute myocardial infarction from twelve Swedish hospitals with coronary care unit Acta Med Scand (Suppl) 586 1975
9 Miettinen O Estimability and estimation in casereferent studies Am J Epidemiol 103 2 226 1976
10 National Center for Health Statistics A summary of studies of interviewing methodology Vital and Health Statistics Series 2 69 1977
11 Rothman K J Causes Am J Epidemiol 104 6 587 1976

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Angina Pectoris in Aortic Valvular Disease and Its Relation to Coronary Pathology

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ABSTRACT Angina pectoris is a common symptom in aortic valvular disease In our study of 100 consecutive patients it was found more commonly in patients with aortic stenosis than in those with aortic insufficiency Only 21 of 80 patients with angina pectoris had significant narrowing (more than 75%) of one or several coronary arteries Angina pectoris in aortic valvular disease thus seems to be most often functional due to disproportion between myocardial oxygen supply and demand On the other hand, 5 of 20 patients without angina pectoris had significant coronary artery stenosis As coronary artery involvement may jeopardize the results of aortic valve replacement in these patients, coronary angiography should always be carried out in patients evaluated for surgery of aortic vavlular disease Coronary bypass surgery should be carried out during the same operation if the stenosis is severe and bypass is technically feasible

Key words angina pectoris aortic valve disease

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Angina pectoris is a common symptom in aortic valvular disease Its mechanism in this condition may be either anatomical due to coronary artery narrowing from atheroma or functional due to disproportion between myocardial oxygen supply and demand In aortic stenosis coronary blood flow and thus oxygen supply may be reduced due to valve pathology with reduction in pressure immediately above the aortic valves or narrowing of aortic orifice due to extensive valvular calcification At the same time oxygen demand is increased due to myocardial hypertrophy which is especially pronounced in aortic stenosis In aortic insufficiency there will be dilatation of the left ventricle with consequent increase in myocardial tension which will increase oxygen demand Furthermore myocardial blood flow may be reduced due to the low diastolic blood pressure

It is important to clarify the coronary anatomy in patients evaluated for aortic valve replacement because Significant coronary artery disease with severe stenosis or occlusion of one or several coronary arteries may jeopardize the surgical results The operative procedure may even in such patients give rise to myocardial infarction Patients with significant coronary artery stenosis should therefore preferably have coronary bypass surgery carried out during the same operation

PATIENTS AND METHODS

We studied 100 consecutive patients with aortic valvular disease evaluated at our department for valve surgery The patients were collected from Jan 1st 1976 to Sept 1st 1977 Following physical examination ECG and plain X ray of the heart the patients were studied with left heart catheterization The function of the left ventricle was evaluated by recording of left ventricular end-diastolic pressure before and after angiography Ejection fraction was calculated from the films taken at cineangiography of the left ventricle and with estimation of its shape and size in the right anterior oblique view Angiography was also carried out with injection above the aortic valves to demonstrate concomitant aortic insufficiency which was classified from 1 to 3 grades Grade 1 slight insufficiency with regurgitation of a small amount of contrast medium into the left ventricle which was immediately emptied Grade 2 moderate aortic insufficiency the contrast agent filled most of the left ventricle which was emptied in one or two beats Grade 3 severe aortic insufficiency the contrast agent filled the whole left ventricle and remained there for several beats

Selective coronary angiography was carried out with preshaped Judkins type catheters (Cordis Corporation Miami) Isopaque® Coronar (Nyegaard Oslo) 3–8 ml was injected into each coronary artery repeatedly as needed by manual pressure Cinerecordings 50 frames/sec 35 mm film often with the addition of 100×100 mm cut films 3–6/sec in multiple projections were obtained Continuous ECG and catheter tip pressure recordings were performed during the procedures

The calculation of percentage area luminal coronary

Table I *Aortic valve systolic gradient incidence of angina pectoris and of significant narrowing of coronary arteries in 100 patients with aortic valvular disease*

	Group I	Group II	Group III
No of pats	44	28	28
Gradient (mmHg)			
Mean	71	71	48
Range	20–150	20–135	0–150
Angina pectoris	40	22	18
Significant coronary stenosis	13	9	4

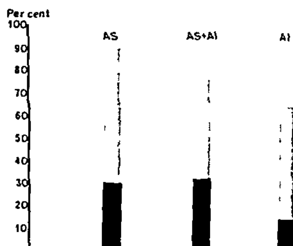


Fig 1 Percentage of angina pectoris (▩) and significant coronary pathology (■) in the various clinical groups *AS* = group I *AS+AI* = group II *AI* = group III

artery stenosis was based upon measuring the luminal diameter in at least 2 projections

Coronary angiographic findings were graded as follows 0 No changes in coronary arteries 1 Up to 50% stenosis of a coronary artery 2 50-75% stenosis 3 75-90% stenosis 4 90-100% stenosis 5 Occlusion of a coronary artery On evaluation of coronary artery changes only those of grades 3 4 and 5 were taken into account Like most other authors we consider stenosis of more than 75% to be significant in relation to the symptoms of angina pectoris

RESULTS

We separated the patients into 3 groups Group I Predominant aortic stenosis pure aortic stenosis or combined with minimal aortic insufficiency Group Combined aortic stenosis and insufficiency of ade 2 (moderate aortic insufficiency) Group III Severe aortic insufficiency (grade 3) either pure or combined with aortic stenosis

As shown in Table I the mean systolic aortic valve gradient was the same in groups I and II (71 mmHg) In group III the mean gradient was lower (48 mmHg)

Angina pectoris was very common 80% of the patients presenting with this symptom Of the patients in group I 90% had angina pectoris against 78% in group II and 64% in group III (Fig 1) Significant stenosis of one or several coronary arteries was found in 13 patients (30%) in group I in 9 (32%) in group II and in 4 (14%) in group III Significant coronary pathology was thus found more often in groups I and II than in group III

Dominance of left coronary artery distribution was found in 19% of this patient series This is a figure higher than normal which is considered to demonstrate 9% dominance of left coronary artery (9)

So far 71 patients have been operated on with aortic valve replacement Eight patients died following operation giving an operative mortality of 11% Of the 8 patients who died only 2 had severe stenosis of one or several arteries

Significant stenosis of one or several coronary arteries was found in 21 of 80 patients with angina pectoris and in 5 of 20 patients without angina pectoris

The distribution of angina pectoris among various age groups is shown in Fig 2 As evident no patient below 50 years of age had significant coronary artery stenosis Coronary pathology was found most commonly in the age groups 60–69 and above 70 years

DISCUSSION

The findings from this study corroborate several previous studies Harris et al (4) found angina pectoris in 58% of their 69 patients above 35 years of age By coronary angiography they observed significant coronary artery changes in 23% Of those with angina 32% had a significant coronary artery obstruction while in the pain free group 10% had significant coronary artery disease In their patients aortic stenosis was the dominant lesion but some patients had trivial aortic regurgitation or mitral valve disease Basta et al (1) observed angina pectoris in 65% of 88 patients with severe aortic valve disease Forty-one of their patients had

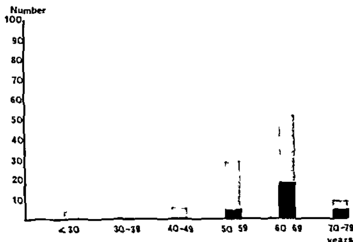


Fig 2 Number of patients with angina pectoris (▣) and significant coronary pathology (■) in the various age groups

predominant aortic stenosis and 10 had severe aortic regurgitation They observed significant coronary artery disease in 25% of those with aortic stenosis and in 20% of those with aortic regurgitation Of 37 patients without angina pectoris coronary arteriography was done in 19 none of whom showed significant coronary artery disease

The results from the study by Hancock (3) are somewhat at variance with the studies above On studying 173 patients with aortic valve disease he considered stenosis of more than 50% to be significant Only patients with predominant aortic stenosis with a pressure gradient of more than 50 mmHg were included He found significant occlusive coronary lesion in 56% of the total group If he had only taken into account stenosis of more than 75% only 35% of his patients would have demonstrated significant coronary artery stenosis He found significant stenosis in 3 of 12 patients below 50 years of age and an increasing incidence with advancing age Significant coronary artery narrowing was found in 30% of patients without angina and here there was also an increase in coronary pathology with advancing age Graboy and Cohn (2) studied 66 patients with severe aortic valvular disease They grouped their patients in the same way as we did predominant aortic stenosis combined stenosis and insufficiency and predominant aortic insufficiency Significant coronary artery disease was found in 21% in group I 28% in group II and 14% in group III Their findings are very similar to ours Linhart et al (7) studied 95 patients with aortic valvular disease They found severe coronary artery disease in 18 patients with aortic stenosis and in 7 with aortic insufficiency Thus 26% of their patients with aortic valve disease had a narrowing of more than 75% of one or several coronary arteries They found angina pectoris in 48% of patients with aortic stenosis and in 20% of patients with aortic insufficiency The incidence of coronary artery disease increased with age Only a few of their patients below 50 years of age had coronary artery disease Mandal and Gray (8) in a series of 60 patients aged 45-66 years with aortic valve stenosis observed angina pectoris in 47% They considered significant coronary stenosis to be present when there was a narrowing of more than 60% of one or more of the main coronary vessels In their series 14 of the 21 patients with angina pectoris had such narrowing of the coronary arteries Two of their patients without angina were subsequently found at autopsy to have severe coronary artery disease They therefore suggested that all patients above the age of 40 with aortic valve disease should be subjected to coronary angiography

In our series significant coronary artery stenosis was found in 25% of the patients more frequently in those with aortic stenosis than in those with aortic insufficiency Significant coronary stenosis was found in only 21 of 80 patients with angina pectoris which was very common in our series It seems therefore that angina pectoris is more often of the functional type in patients with aortic valve disease On the other hand 5 of 20 patients without angina pectoris had significant coronary artery disease Coronary angiography should therefore be carried out in all patients with aortic valve

disease who are being considered for aortic valve replacement

We also would like to draw attention to the fact that 19% of our patients had dominance of the left coronary artery system Murphy et al (9) in a study of 75 patients with aortic stenosis and a control group of 150 patients found left coronary artery dominance in 25% of those with aortic stenosis and in 9% of the control patients The difference was significant In patients with aortic stenosis they observed that the left main coronary artery was shorter in those with left than with right dominance They further observed a higher incidence of perioperative myocardial infarction in those with left dominance (27%) than with right or balanced coronary artery distribution (7%) They considered dominant left coronary artery system to be an associated congenital defect in patients with aortic stenosis Similarly Johnsen et al (6) found a short left main coronary artery and left coronary dominance in 27% of patients with bicuspid aortic valves and in 12% of a control group Higgins and Wexler (5) found an even higher incidence of left coronary artery dominance in children with bicuspid aortic valves (56 8%) and in adults with isolated aortic stenosis (36%)

REFERENCES

1 Basta L L Raines D Najjar S & Kioschos J M Clinical haemodynamic and coronary angiographic correlates of angina pectoris in patients with severe aortic valve disease Br Heart J 37 150 1975
2 Graboy T B & Cohn P T The prevalence of angina pectoris and abnormal coronary arteriograms in severe aortic valvular disease Am Heart J 93 683 1977
3 Hancock E W Aortic stenosis angina pectoris and coronary artery disease Am Heart J 93 382 1977
4 Harris C N Kaplan M A Parker D P Dunne E F Cowell H S & Ellestad M H Aortic stenosis angina and coronary artery disease Br Heart J 37 656 1975
5 Higgins C B & Wexler L Reversal of dominance of the coronary arterial system in isolated aortic stenosis and bicuspid aortic valves Circulation 52 292 1975
6 Johnson A D Detwiler J H & Higgins C B Left coronary artery anatomy in patients with bicuspid aortic valves Br Heart J 40 489 1978
7 Linhart J W de la Torre A Ramsey H W & Wheat M W The significance of coronary artery disease in aortic valve replacement J Thorac Cardiovasc Surg 53 811 1968
8 Mandal A B & Gray J R Significance of angina pectoris in aortic valve stenosis Br Heart J 38 811 1976
9 Murphy E S Rosch J Starr A & Rahimtoola S H Frequency and significance of left coronary artery dominance in isolated aortic stenosis Clin Res 24 87 1976

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Oesophageal Dysfunction in Non-Infarction Coronary Care Unit Patients

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ABSTRACT Oesophageal dysfunction (OD) is a common finding in patients discharged from a coronary care unit without definite diagnosis *Of 55 patients* investigated with oesophageal manometry, acid perfusion test and exercise ECG 32 had signs of OD and 19 signs of ischaemic heart disease (IHD) Symptoms *such as heart burn, acid regurgitations,* feeling of a lump in the throat, surfeitness after meals, chest pain at night, and relief of chest pain when lying with the head raised were significantly more common in patients with OD than in patients with normal oesophageal function Chest pain was significantly more often provoked by effort emotions or cold and more often relieved by nitroglycerine in patients with signs of IHD than in those without These pain provoking factors were, however, also common in patients with OD A careful case history with specific inquiry directed at not only cardiac but also oesophageal symptoms is important in the differential diagnosis of chest pain

Key words acid perfusion test coronary care unit effort angina ischaemic heart disease oesophageal manometry

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In about half of all patients admitted to coronary care units (CCU) acute myocardial infarction is diagnosed *while the remaining half is discharged* with other diagnoses (13) The majority of the latter group has previously suffered a myocardial infarction (MI) and there is reason to believe that the current episode is also of cardiac origin *Others* have obvious extracardiac diagnoses e g pulmonary emboli pneumothorax pleuritis or cholecystitis A considerable number of patients are however discharged without a definite diagnosis Bennett and Atkinson (3) found an incidence of 64% of reflux oesophagitis as diagnosed by oesophagoscopy in patients with chest pain who were urgently admitted to a medical ward but did not develop MI Similar Swedish patient materials comparable from the oesophageal point of view are lacking but our experience from other patients with chest pain (1 14 16) made us believe that oesophageal dysfunction may also be a common problem in Swedish CCUs

Our aim was to investigate the incidence of oesophageal dysfunction (OD) and ischaemic heart disease (IHD) in non-infarction CCU patients and to relate symptoms to objective findings

PATIENTS

During a six month period 390 patients with acute chest pain were admitted to the CCU Admission criterion was pain of a duration of 30 min or more and of a character which gave suspicion of acute MI The discharge diagnosis was acute MI in 196 patients Another 104 patients had previously suffered MI and in six coronary artery disease had previously been diagnosed by coronary *angiography Fifteen* patients were diagnosed as having chest pain of origin other than cardiac or oesophageal

The remaining 69 patients had no definite diagnosis when discharged and were considered for the present study Ten were excluded because of orthopaedic or mental handicap or age exceeding 75 years The remainder 59 patients were invited to join the investigation four patients declined

The patient series therefore comprises 55 non infarction patients 38 men (mean age 56 5) and 17 women (mean age 58 1) all giving their informed consent The age and sex distribution is given in Fig 1

In no case did the ECG at rest show signs of MI but it was abnormal in other ways in 24 of the 55 patients One *patient had left (LBBB) and three had right bundle branch* block (RBBB) two had signs of left ventricular hypertrophy and four had arrhythmias Unspecific ST-T changes were seen in 14 patients five of whom were on digitalis therapy

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Abbreviations CCU=coronary care unit IE-ECG=ischaemic exercise electrocardiogram IHD=ischaemic heart disease LES=lower oesophageal sphincter MI=myocardial infarction OD=oesophageal dysfunction LBBB=left bundle branch block RBBB=right bundle branch block

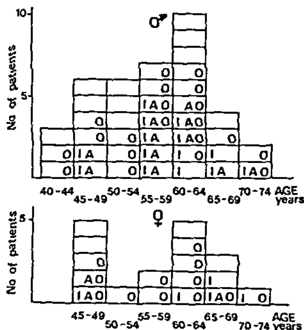


Fig 1 Frequency of ischaemic exercise ECG (*I*) effort angina at exercise test (*A*) and oesophageal dysfunction (*O*) in 38 male and 17 female non infarction CCU patients Empty box indicates normal finding

METHODS

Within 2–6 months after discharge the patients were investigated with a set of oesophageal function tests and a graded exercise test with continuous ECG recording They were also asked to fill in a questionnaire regarding ymptoms of possible oesophageal or cardiac (10) origin

The oesophageal function was tested by manometry and by an acid perfusion test The oesophageal manometry included investigation of tonus of the lower oesophageal sphincter (LES) LES displacement and gastro-oesophageal reflux by abdominal compression and a motility test (12) The acid perfusion test was slightly modified from the original Bernstein test (1 4) The test was considered positive if the patient experienced pain or heart burn upon infusion of a solution of 0 1 M HCl into the oesophagus and the result of the test could be reproduced three times within half an hour

The patients were classified as having OD if at least one of the following four criteria were met 1 Positive acid perfusion test 2 Manometrically verified hiatal hernia with a length of 2 cm or more without abdominal compression 3 Dysmotility (i e simultaneous contractions at a length of 10 cm at dry swallowing in two of ten tested levels in the oesophagus) combined with hypotonia of the LES (i e a pressure gradient of less than 9 mmHg between the LES and the oesophagus at the end of expiration) or combined with oesophageal reflux as diagnosed by a decrease in pH in the distal part of the oesophagus upon application of an extraabdominal pressure of 100 mmHg 4 Severe dysmotility (i e simultaneous contractions at a length of 10 cm at dry swallowing in at least three of ten tested levels in the oesophagus) Hypotonia of the LES or gastro-oesophageal reflux as a single finding was not classified as OD

A 12 lead ECG was registered at rest and evaluated according to the Minnesota code (11) Sitting exercise was performed on an electrically braked bicycle ergometer with stepwise increase of loads every 6 min using pain or fatigue as end point The patients were asked to abstain from smoking eating major effort or nitro intake for four hours prior to the test The ECG was registered recumbent before during 10 min after the exercise (extremity + V leads) and continuously during exercise with the forehead as reference (CH_{1-6})

The ECG changes during exercise were classified according to Areskog et al (2) ST changes during work being classified from 0 to 3 points Changes within 0 1 mV were scaled as 0 and a junctional ST depression of 0 3 mV or more with form changes as 3 T wave changes after exercise were also scaled from 0 to 3 0 denoting minor changes after exercise with successive restitution of the T wave amplitude and 3 a transient change of the T wave amplitude to less than −0 2 mV within 2–4 min after exercise without parallelity to decrease in heart rate The sum of the ST and T wave scaling was calculated A typical ischaemic ECG reaction at the exercise test (IE–ECG) was defined as 2–6 points for patients without digitalis therapy and 3–6 points for patients receiving digitalis

For the purpose of the present study the diagnosis of IHD was based on the finding of IE–ECG in 17 patients LBBB in one and RBBB + left anterior hemiblock in one

Chest pain in connection with the exercise test was classified by a trained physician from 0 to 3 considering character site and time course 0=no pain 1=atypical pain 2=almost typical pain 3=typical anginal pain In the further presentation groups 0–1 will be referred to as atypical and groups 2–3 as typical anginal chest pain

Fisher s exact test was used for statistical evaluation

RESULTS

Exercise ECG and oesophageal function tests

Thirty two patients (58%) had signs of OD (Table I) and 19 (35%) of IHD ten of them in combination with OD Fourteen patients had neither signs of OD nor IHD The outcome of the tests in relation to age and sex is displayed in Fig 1 Fifteen patients (27%) experienced typical effort angina at the exercise test Of these six had signs of IHD seven of IHD + OD and two of OD alone (Fig 2) Forty patients experienced no or atypical pain at the exercise test Of these three had signs of IHD three both of IHD and OD and 20 of OD alone Fourteen patients had normal findings

Questionnaires (Table II)

Patients with OD had a significantly higher incidence of heart burn acid regurgitations feeling of a

Table I *Findings at oesophageal function tests*

	Dysmotility	LES hypotonia	Positive acid perfusion test	Hiatal hernia
OD (n=32)	28	16	15	15
Non OD (n=23)	4	4	0	0
Total (n–55)	32	20	15	15

lump in the throat surfeitness after meals hacking cough chest pain at night and relief of chest pain when lying with their head raised than patients with normal oesophageal function In patients with OD and hacking cough smoking was significantly more frequent (8/14) than in the OD group without hacking cough (4/18 $p<0\ 05$) Patients with signs of IHD had a significantly higher incidence of positive Rose questionnaire (10) had more often pain in duced by cold or emotions and relief of chest pain by nitroglycerine than patients with normal ECG reaction at the exercise test

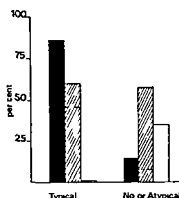


Fig 2 Frequency of IHD (■) and OD (▨) in 15 patients with typical and 40 with no or atypical chest pain at exercise test □=Normal finding ---= proportion of pa tients with a combination of IHD and OD

DISCUSSION

The patients were admitted to a CCU because of chest pain giving suspicion of MI but discharged without any diagnosis in spite of routine differential diagnostic efforts At the follow up test 2–6 months later 58% had signs of OD and 35% signs of IHD The high frequency of OD is in accordance with the results of Bennett and Atkinson (3) in a similar patient group We have also found a high incidence of OD in other patient series selected because of chest pain (1 14 16) all in contrast to the findings in a male population sample *(12)*

There is no doubt that severe chest pain can be induced from the oesophagus (1 5 8 9) However the question arises if it was OD that caused the pain which brought some of the patients to hospital The

Table II *Questionnaire replies in relation to outcome of the function tests*

	OD (n=32)	Non-OD (n=23)	IHD (n=19)	Non IHD (n=36)
Do you often have heart burn?	11*	2	3	10
Do you often have acid regurgitations?	11*	2	4	9
Do you often feel a lump in your throat?	21	4	9	16
Do you often feel surfeited after a meal?	16 *	3	6	13
Do you often have a hacking cough?	14 **	1	7	8
Does your chest pain get worse when lying down?	10	3	5	8
Does your chest pain relieve when lying with your head raised?	13	3	6	10
Do you sometimes wake up at night through chest pain?	21	5	12	14
Do you sometimes get chest pain in cold environments?	15	7	11*	11
Do you get chest pain with emotional distress?	23	12	16*	19
Does your chest pain get worse in connection with effort?	24	13	16	21
Rose questionnaire positive	19	10	14	15
Have you ever taken nitroglycerine?	12	8	13***	7
If yes did the pain relieve?	8	6	12	2
Effort angina at exercise test	9	6	13***	2
IHD	10	9		
OD			10	22

*$p<0\ 05$ $p<0\ 01$ $p<0\ 001$

present patient group was selected when common differential diagnoses had been excluded during admission Therefore our working hypothesis is that the finding of OD or IHD at the 2-6-month follow up was related to the chest pain which brought the patients to hospital Naturally it would be desirable to make this distinction earlier and if possible from the history alone during the CCU stay

Symptoms such as heart burn acid regurgitations feeling of a lump in the throat surfeitness after meals chest pain at night and relief of chest pain when lying with the head raised were significantly more common in patients with OD than in those with normal oesophageal function and seem to be of diagnostic value Chest pain was significantly more often provoked by effort emotions or cold in patients with signs of IHD than in those without as was also the finding of a positive Rose questionnaire These latter findings are however of limited value in the diagnosis of the individual patient since the same pain provoking factors were also common in patients with signs of OD only Similar findings were reported by Bennett and Atkinson (3) Patients with signs of IHD reported relief of pain after intake of nitroglycerine more often than those without This suggests that the response to nitroglycerine may be of differential diagnostic value It should be borne in mind however that patients with oesophageal spasm also respond favourably to nitroglycerine administration (15) but with another time course than patients with IHD (7 9)

Typical effort angina observed at the exercise test was a common finding in patients with signs of IHD but not in patients with OD only The value of observing chest pain in connection with an exercise test in the diagnosis of IHD is in accordance with our earlier experience from a group of patients in whom the diagnosis of IHD was based on coronary angiogram (14) results which also are supported by findings of Areskog et al (2) and by Cole and Ellestad (6)

In conclusion we have found a high incidence of OD in a group of patients discharged from a CCU without definite diagnosis The symptomatology as registered in a questionnaire was significantly correlated to the outcome of the tests A careful case history with specific inquiry directed at not only cardiac but also oesophageal symptoms is important in the differential diagnosis of chest pain

ACKNOWLEDGEMENTS

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REFERENCES

1 Areskog M Tibbling L & Wranne B Oesophageal acid perfusion test as a complement to work test in patients with chest pain Acta Med Scand 201 559 1977
2 Areskog N H Bjork L Bjork V O Hallén A & Strom G Physical work capacity Ecg reaction to work test and coronary angiogram in coronary artery disease Acta Med Scand (Suppl) 472 9 1967
3 Bennett J R & Atkinson M The differentiation between oesophageal and cardiac pain Lancet 2 1123 1966
4 Bernstein L M & Baker L A A clinical test for oesophagitis Gastroenterology 34 760 1958
5 Brand D L Martin D & Pope C E Esophageal manometrics in patients with angina like chest pain Dig Dis 22 300 1977
6 Cole J P & Ellestad M H Significance of chest pain during treadmill exercise correlation with coronary events Am J Cardiol 41 227 1978
7 Henderson R H Wigle E D Sample K & Maryatt G Atypical chest pain of cardiac and esophageal origin Chest 73 24 1978
8 Kramer P & Hollander W Comparison of experimental esophageal pain with clinical pain of angina pectoris and esophageal disease Gastroenterology 29 719 1955
9 Roberts R Henderson R D & Wigle E D Esophageal disease as a cause of severe retrosternal chest pain Chest 67 523 1975
10 Rose G A The diagnosis of ischaemic heart pain and intermittent claudication in field surveys Bull WHO 27 645 1962
11 Rose G A & Blackburn H Cardiovascular survey methods WHO Geneva 1968
12 Spandow O Sokjer H & Tibbling L Function of the lower oesophageal sphincter in a population selected at random A manometric radiological and questionnaire study Acta Otolaryngol 78 295 1974
13 Sawe U Early diagnosis of acute myocardial infarction with special reference to the diagnosis of intermediate coronary syndrome Acta Med Scand (Suppl) 545 9 1972
14 Svensson O Stenport G Tibbling L & Wranne B Oesophageal function and coronary angiogram in patients with disabling chest pain Acta Med Scand 204 173 1978
15 Swamy N Esophageal spasm clinical and manometric response to nitroglycerine and long acting nitrites Gastroenterology 72 23 1977
16 Tibbling L & Wranne B Oesophageal dysfunction in male patients with angina like pain Acta Med Scand 200 391 1976

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Micromethods for Analysis of Lipids in Endomyocardial Biopsy Specimens

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ABSTRACT Triglycerides and phospholipids extracted from endomyocardial biopsy specimens were quantitated with sensitive photometric methods and the fatty acid composition of phospholipids was determined after separation of the methyl esters by gas chromatography The degree of triglyceride accumulation was expressed as the molar ratio triglyceride/phospholipid since in most cases the phospholipid concentration is relatively constant The representativity of the analysis of endomyocardial biopsy was investigated by comparing lipid analyses of large and small myocardial specimens obtained at necropsy The methods described may be valuable tools in studies on metabolic disturbances in different cardiomyophaties

Key words endomyocardial biopsy triglyceride phospholipid fatty acid cardiomyopathy

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A better understanding of the pathogenesis of cardiomyopathy requires a classification on a biochemical basis Recent methods for endomyocardial biopsy permit various kinds of biochemical analysis (10 11) but it is not yet known what diagnostical information such analysis can provide Determination of the lipid content and composition of the myocardium is of interest since lipid deposition is known to occur in several pathological conditions affecting the heart (4 5 7) Due to the small amounts of tissue which can be obtained with endomyocardial biopsy methods with a high sensitivity are required This communication briefly presents the development of such methods and demonstrates the applicability of these methods for the analysis of triglyceride and phospholipid content and phospholipid fatty acid composition of endomyocardial biopsy specimens

MATERIAL AND METHODS

Endomyocardial biopsy was performed as described previously (10) on patients admitted to the hospital for diagnostic investigation of heart disease of various origin The biopsy specimens (1–5 mg wet weight) were washed in saline and immediately frozen to −20 C and kept at this temperature until further analysis For a study of the representativity of lipid analysis in individual biopsy specimens larger pieces (0 4–0 9 g) of myocardium as well as adjacent pieces the same size as the biopsy specimens were collected from necropsies within 15 h after death These samples were collected from the region in the septal apical area in the right ventricle where endomyocardial biopsy in vivo is performed The corpses had been stored at +4 C This material was also frozen at −20°C until further use After removal of connective tissue the myocardial tissue was lyophilized and the dry weight was sometimes determined The lipids were extracted with chloroform methanol (2 1) and an anhydrous chloroform extract was prepared essentially as previously described for lipid analysis of liver biopsy specimens (3) Phospholipids (PL) were determined as lipid phosphorus in the extracts (3 12) and after removal of PL by adsorption to Florisil the triglycerides (TG) were determined as glyceride glycerol with an enzymatic fluorimetric method (3 12) With the larger pieces of myocardium the method had to be scaled up appropriately otherwise the same procedure was followed

The Florisil with adsorbed PL was washed twice with chloroform and was then transferred to tubes with ground glass stoppers with 2×100 µl 2% H_2SO_4 in methanol or a correspondingly larger volume with the larger pieces After transestenfication at 65 C for 4 h water was added and the methyl esters were extracted three times with hexane and analysed with gas chromatography (1) A system with ethyleneglycolsuccinate polyester as a stationary phase was used Model studies indicated that all major PL were degraded under these conditions

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[1] Research Fellow in Nutrition sponsored by the Swedish Medical Research Council (project no 4931)

Abbreviations TG = triglycerides PL = phospholipids TG/PL = molar ratio triglycerides/phospholipids

Table I *Comparison of TG/PL between large and small samples of myocardia obtained at necropsy*
Causes of death arteriosclerotic heart disease 4 disseminated malignant disease 2

Necropsy no	Large samples: Wet weight (g)	PL[a] (nmol/mg wet weight)	TG/PL[a]	Small samples (wet weight <5 mg): TG/PL[b]	n
1	0 713	19 2	0 22	0 24 ±0 034	6
2	0 689	17 9	0 057	0 064±0 006	8
3	0 693	19 7	0 24	0 27 ±0 018	9
4	0 381	20 5	0 24	0 20 ±0 023	5
5	0 538	22 5	0 073	0 054±0 004	6
6	0 883	18 5	0 28	0 31 ±0 049	6

[a] Mean of three determinations [b] Mean ± S E M

RESULTS AND DISCUSSION

Determination of molar ratio triglycerides/phospholipids (TG/PL) The biopsy specimens were quite small and variations in water content due to evaporation made it difficult to weigh them accurately Therefore TG concentration was better expressed in relation to PL The PL concentration does not appear to change appreciably during lipid deposition in the myocardium (2 10) (Tables I and II) as shown also for other organs e g the liver (12) Thus we have used the TG/PL as an approximate measure of myocardial TG accumulation

As shown in Table I there was a high degree of correlation (r=0 97 p<0 001) between the average TG/PL in large and small pieces from one and the same myocardium This seemed to be the case irrespective of the degree of TG accumulation The PL concentration was relatively constant despite an almost five fold variation of TG content validating the use of the TG/PL as a measure of TG accumulation The PL concentrations obtained were similar to those reported by others (2 6) Table I also indicates that there was a considerable variation in the TG/PL of individual small samples This variation was larger than that obtained in similar determinations in liver specimens (12) possibly due to a less homogeneous distribution of TG in the myocardium

Determinations of TG and PL were performed in the same way on endomyocardial biopsy specimens from four patients (Table II) The data illustrate that the method employed was sensitive enough to allow determination of TG and PL even in small endomyocardial biopsy specimens with a relatively low content of TG as indicated by the TG/PL Approximate weights of the lyophilized specimens were obtained and the PL concentrations calculated to again illustrate the relative constancy of this parameter despite a widely varying TG content

Table II *TG/PL and PL concentrations in endomyocardial biopsy specimens from four patients*

Pat no	TG/PL	Dry weight[a] (mg)	PL[b] (nmol/mg dry weight)
1	0 062	0 5	143
2	0 023	0 5	133
3	0 078	0 7	157
4	0 119	1 2	133

[a] Obtained from lyophilized samples [b] Mean of two determinations

Table III *Analysis of fatty acid composition in small PL samples*

Different amounts of egg lecithin were transesterified in 2% H_2SO_4 in methanol and the methyl esters were analyzed by gas chromatography data are expressed as weight %

Fatty acid	Sample size (µg): 500[b]	90	18	18
16 0	32 9±0 4[b]	32 3	31 3	30 8
16 1	1 4±0 1	1 3	1 4	1 4
18 0	15 3±0 1	16 0	16 1	16 3
18 1	32 0±0 2	32 2	32 9	32 9
18 2	15 4±0 4	16 1	15 0	15 9
20 4	3 0±0 2	2 1	3 4	2 7

[a] No of carbon atoms no of double bonds
[b] Mean ± S E M (n=4)

Table IV *Comparison of PL fatty acid composition in multiple biopsies from one and the same heart obtained at necropsy*

Data are expressed as weight %

Fatty acid	Large biopsy[b]	Small biopsies A	B	C
16 0	17 9	21 1	19 1	17 3
16 1	0 4	0 0	0 1	0 3
18 0	15 2	18 4	16 0	14 2
18 1	14 2	15 1	16 1	13 7
18 2	27 1	22 1	25 8	28 1
20 4	16 9	16 8	15 4	15 6
22 6	8 6	6 6	7 6	11 2

No of carbon atoms no of double bonds
[b] Mean of four runs
Mean of two runs

The PL concentrations obtained in this way seem to be higher than those found in the large necropsy samples (Table I) or reported by others (2 6) but since water content and other factors in the lyophilized specimens were not controlled a direct comparison should probably not be made

Determination of fatty acid composition of heart PL Model experiments showed that the fatty acid composition of small amounts of lecithin could be accurately determined by the method used (Table III) The same findings were obtained for other major PL The experiments described in Table IV were performed to study the representativity in the determination of the fatty acid composition of PL from a single biopsy specimen One large and several small heart samples taken from the same heart at necropsy were analyzed In a preliminary experiment some variation was encountered between samples especially in the proportion of palmitic acid This probably reflected contamination with non myocardial tissue since the variation was considerably smaller when contaminating tissue had been more carefully cut off from the myocardial samples (Table IV) Table V shows analyses from biopsy specimens taken from two patients with cardiomyopathy Further studies are necessary to evaluate the possible pathophysiological meaning of the differences between the patients

Table V *Fatty acid composition of total PL from human myocardial biopsies*

Patient	Fatty acid composition (weight %) 16 0	16 1	18 0	18 1	18 2	20 4	22 6
A	15 5	0 5	16 5	12 9	21 0	25 4	8 3
B	[illegible]2 0	–	19 2	16 2	21 0	21 6	12 5

Phosphatidylcholine (lecithin) and phosphatidyl ethanolamine are the major PL in the human heart (6) The fatty acid composition in total PL found by us is well compatible with Fletcher s data (6) since it is intermediary between that of phosphatidylcholine and phosphatidylethanolamine Diet would be expected to influence the fatty acid composition Szuhaj and McCarl (9) noted that dietary fatty acids influenced mainly the neutral lipids and to a lesser extent the polar lipids The neutral lipid amount in our samples was too small to permit analysis of fatty acid composition

CONCLUSION

This study demonstrates the feasibility of determining the amount and fatty acid composition of lipids in endomyocardial biopsy specimens with sensitive micromethods Such procedures are valuable tools in further studies on metabolic disturbances in various heart diseases

ACKNOWLEDGEMENT

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REFERENCES

1 Åkesson B Elovson J & Arvidson G Initial incorporation into rat liver glycerolipids of intraportally injected [^{3}H]glycerol Biochim Biophys Acta 210 15 1970
2 Alavaikko M Elfving R Hirvonen J & Jarvi J Triglycerides cholesterol and phospholipids in normal heart papillary muscle and in patients suffering from diabetes cholelithiasis hypertension and coronary atheroma J Clin Pathol 26 285 1973
3 Belfrage P Wiebe T & Lundquist A Methods for the determination in the nanomol range of lipids in liver fine needle aspiration biopsies Scand J Clin Lab Invest 26 53 1970
4 Blieden L C & Moller J H Cardiac involvement in inherited disorders of metabolism Prog Cardiovasc Dis 16 615 1974
5 Ferrans V J Hibbs R G Weilbaecher D G Black W C Walsh J J & Burch G E Alcoholic cardiomyopathy A histochemical study Am Heart J 69 748 1965
6 Fletcher R F Lipids of human myocardium Lipids 7 728 1972

7 Hibbs R G Ferrans V J Black W C Weilbaecher D G Walsh J J & Burch G E Alcoholic cardiomyopathy An electron microscopic study Am Heart J 69 766 1965
8 Lindlar F & Zaki I A Lipidchemische Unter suchungen zur Frage der degenerativen Myokardver fettung Virchows Arch (Pathol Anat) 341 142 1966
9 Szuhaj B F & McCarl R L Fatty acid composi tion of rat hearts as influenced by age and dietary fatty acids Lipids 8 241 1973
10 Torp A Endomyocardial biopsy Scand J Thorac Cardiovasc Surg 7 253 1973
11 — Cardiomyopathy A hemodynamic and microscop ical study on a clinical material using endomyocardial biopsy Dissertation University of Lund Lund Sweden 1974
12 Wiebe T & Belfrage P Human liver lipids rep resentativity of determinations on material obtained by fine needle aspiration biopsy Scand J Clin Lab Invest 28 453 1971

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Circumstances around the Onset of a Myocardial Infarction

A Study of Factors Relevant to the Perception of Symptoms and to the Delay in Arriving at a Coronary Care Unit

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ABSTRACT Psychosocial factors, experienced pain and anxiety in relation to patient delay were studied in 100 patients admitted for suspected acute myocardial infarction (AMI) More severe pain was reported by women, by those who had recently consulted a physician, who experienced severe anxiety, who fell ill away from their place of work, or who possessed little medical knowledge These patients tried to get relief from pain by resting Patients experiencing more severe anxiety were younger, had not consulted a physician recently had poor medical knowledge, belonged to lower socio-economic groups or were impatient These patients also sought relief from pain by resting Pain, but not anxiety, was related to delay Long delay was seen more often in patients who did not believe they had suffered an AMI and who were psychologically inactive prior to the onset of pain Recent physician consultation failure to call for help and belonging to lower socio-economic groups were also related to long delay Medical knowledge was unrelated to patient delay Patients with a low degree of pain rarely reported considerable anxiety whereas several patients with severe pain had little or no anxiety

Key words myocardial infarction delay pain anxiety psychosocial factors

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Many patients die of acute myocardial infarction (AMI) in the first hours after the onset of symptoms before reaching hospital This high incidence of early deaths is due in part to an unfortunately long delay before seeking medical help The most important factor behind the total delay before admission to a coronary care unit (CCU) concerns the patient himself—the time it takes to decide to seek medical help i e patient delay (2) In a previous study (3) we found that patient delay was more than one hour in 60% of the patients admitted to a CCU in Stockholm and the median time for total delay was 3–4 hours

Reduction of patient delay with quicker admission to hospital might be one way of reducing early deaths in AMI Our knowledge of the psychological and social factors as well as our comprehension of the two major subjective sensations—pain and anxiety—and how these affect patient delay are however limited Therefore we have attempted to clarify the importance of some psychological and social factors in relation to patient delay with special reference to a subjective grading of pain and anxiety

PATIENTS AND METHODS

One hundred patients 63 men and 37 women with a mean age of 64 years (range 35–80) admitted to our CCU in 1976 for acute and well defined onset of central chest pain were included in the study AMI was verified in 81 of the patients whereas 19 had acute chest pain due to angina pectoris or of other origin Interviews were performed within 48 hours of admission by two nurses who checked one another for consistency of judgement during the first ten interviews

Delay The variables included were Age Sex Psychological and physical activity at onset of pain Who took the initiative in calling for help? Who actually called for help? Was help resisted by the patient? To whom was the call for help directed? Was action taken to relieve pain? Was medicine taken to relieve pain? What was the effect of medicine on the pain? Degree of pain anxiety psychological impatience and medical knowledge Occupation Whereabouts at onset of pain Was someone else

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Abbreviations AMI=acute myocardial infarction CCU=coronary care unit IHD=ischaemic heart disease

Table I *Factors affecting long and short delay*
Only variables with a squared beta coefficient of ≥0 01 in the long delay category have been listed

	Long delay (>6 h n=37)			Short delay (<2 h n=52)		
	Squared beta	Squared eta	Direction in multivariate analysis	Squared beta	Squared eta	Direction in multivariate analysis
Patient s own diagnosis was MI	0 15	0 13	–	0 09	0 11	+
Psychological activity before onset	0 07	0 08	–	0 04	0 05	+
High professional group	0 04	0 01	–	0 02	0 00	+
Not consulted physician recently	0 03	0 04	–	0 01	0 05	+
Patient called for help	0 03	0 00	–	0 01	0 00	+
High degree of pain	0 03	0 02	–	0 01	0 01	+
Ingested heart medication for relief	0 03	0 00	–	0 00	0 03	+
Presence of another person	0 02	0 04	–	0 00	0 00	+
High degree of anxiety	0 02	0 04	–	0 01	0 01	+
Initiative by patient himself	0 02	0 00	–	0 02	0 00	+
High age	0 02	0 08	+	0 06	0 04	–
High degree of impatience	0 02	0 00	+	0 12	0 04	–
Male sex	0 01	0 03	–	0 02	0 02	+
Previous history of CCU care	0 01	0 08	–	0 02	0 08	+
Called correct agency	0 01	0 01	+	0 00	0 01	+
Attempts to relieve pain by resting	0 01	0 00	+	0 00	0 00	+

present? Previous diseases Call to a doctor in the last year Diagnosis (AMI/non AMI)

The methods used to grade pain anxiety impatience and medical knowledge require further explanation

Pain and *anxiety* in relation to the acute episode were measured by the graphic rating method of Huskisson (6) A horizontal line was shown to the patient The patient was instructed to think of the degree of pain and anxiety at he had experienced at onset of symptoms and to mark is on the line considering that one end corresponded to no pain/anxiety and the other end to unbearable pain/anxiety For quantification the line was divided into 20 sections

The degree of *psychological impatience* was estimated from three questions 1) Do you feel hostile when held up in queues? 2) Do you feel hostile when talking to a slow person who never gets to the point ? 3) Do you feel in a hurry even when there is plenty of time? A yes answer scored two points a yes somewhat one and no zero point The sum of scores provided an impatience score which is related to the impatience (I) dimension of the JAS type A score (7)

Medical knowledge was estimated by (*a*) the patient s ability to correctly locate the position of the heart liver thyroid stomach and kidney on a drawn human figure and (*b*) specific questions regarding ischaemic heart disease (IHD) The patients were divided arbitrarily into three groups with good adequate and poor medical knowledge Here as elsewhere the patients were classed into groups so constructed that there would be adequate numbers in each

Statistical analysis

Multiple non linear analysis was performed This program allows both univariate and multivariate analysis of categorized non linear non dependent and dependent variables The method used is based on a generalization of that described by Andrews et al (1) A great squared beta coefficient indicates a strong association of the non dependent variable with the dependent when all other variables have been taken into account (multivariate analysis) Since the dependent variable is non linear the squared beta coefficient has to be given for each category of the dependent variable separately The squared eta coefficient corresponds to the amount of variance explained by the variable when the other non dependent variables have not been taken into account (univariate analysis)

Correlation coefficients were calculated according to Goodman and Kruskal (4) A coefficient of 1 0 indicates complete association between the rank orders of the two variables in a pair Values of 0 40–0 69 indicate a moderate and values of 0 70–0 99 a strong association

The following categories were used for the dependent variables *Delay* short (<2 hours) medium (2–6 hours) long (>6 hours) *Pain* (score 0–20) minimal (0–13) moderate (14–16) maximal (≥17) *Anxiety* (score 0–20) minimal (0–2) moderate (3–13) maximal (≥14)

RESULTS

Associations between variables

Correlations were computed for all 253 possible pairs of single variables and 24 correlation coefficients of ≥0 40 were found

Table II *Degree of pain in relation to various factors*

	High grade pain (≥17 n=44)			Low grade pain (≤14 n=32)		
	Squared beta	Squared eta	Direction	Squared beta	Squared eta	Direction
Attempts to relieve pain by resting	0 13	0 04	+	0 00	0 01	–
Not consulted physician recently	0 05	0 01	–	0 01	0 01	+
High degree of anxiety	0 05	0 04	+	0 04	0 05	–
Onset of pain at workplace	0 04	0 01	–	0 03	0 02	+
Male sex	0 03	0 03	–	0 06	0 04	+
Good medical knowledge	0 03	0 06	–	0 01	0 03	+
Presence of another person	0 02	0 02	+	0 01	0 01	–
Previous CCU care	0 02	0 01	–	0 00	0 01	0
Resisted aid	*0 02*	*0 01*	*+*	*0 00*	*0 00*	+
Relief from medication	0 01	0 01	+	0 01	0 00	–
High professional group	0 01	0 01	–	0 03	0 01	–
Psychological activity at onset	0 01	0 00	+	0 02	0 01	–

Onset away from both home *and* workplace showed a reverse association with degree of pain

Strong correlations (>0 85) were observed between psychological and physical activity at onset between another person taking the initiative and another person calling for help between taking heart medication for relief and obtaining relief as well as between having consulted a physician recently (particularly for IHD) and previous CCU care

Moderate correlations (0 40–0 80) were also observed for several clusters of associations Thus physical or psychological activity at onset tended to occur more frequently among those whose onset took place away from home Whenever some other person was present at onset this subject took the initiative and called for suitable help more frequently than the subject himself The variables previous CCU care having consulted a physician recently having ingested heart medication at onset of symptoms and feeling relief from this were also intercorrelated Male patients tended to resist aid more frequently than female patients Older subjects had their disease onset at home to a greater extent than younger

Delay

The patients who were most likely to have a *long delay* were those who 1) did not initially believe that they had suffered a myocardial infarction 2) had not been psychologically active prior to onset of pain 3) belonged to the lower socio-economic strata 4) had consulted a physician recently 5) did not call for help themselves and 6) reported a low degree of pain

Those who were most likely to have a short delay essentially belonged to the opposite groups (Table I) One variable high degree of impatience was an exception It was associated with decreased likelihood of belonging to the short delay group Table I also illustrates that several variables which seemed to be good predictors according to the univariate analysis proved bad according to multivariate analysis an example being previous CCU care which was an important predictor of short delay Utilizing *all* the non dependent variables 78% of the cases were correctly classified in the three delay groups

Pain

Severe pain was thus related to short delay (Table II) Those who were most likely to report severe pain were patients who had 1) attempted to relieve pain by resting 2) consulted a physician recently 3) high degree of anxiety 4) onset away from place of work 5) female sex and 6) poor medical knowledge

Those who reported little pain essentially belonged to the opposite groups Little pain was also reported by those who had onset of pain during work Utilizing all non dependent variables 68% of all subjects were correctly classified with regard to maximal medium and minimal pain

Anxiety

Anxiety was related to pain but not to delay (Table III) Patients with a high degree of anxiety were those who 1) had not consulted a physician recent

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High degree of impatience	0 02	0 00	+	0 12	0 04	–
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Correlations were computed for all 253 possible pairs of single variables and 24 correlation coefficients of ≥0 40 were found

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Rapid Thrombolysis and Preservation of Valvular Venous Function in High Deep Vein Thrombosis

A Comparative Study between Streptokinase and Heparin Therapy

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ABSTRACT The results of streptokinase and heparin treatment are compared in a 4-year prospective study with special reference to preservation of high valvular venous function An objective assessment was based upon phlebographic examinations before, during and 1–2 months after therapy Complete lysis was demonstrated in 44 % of high thromboses treated with streptokinase and in 6 % treated with heparin Retrograde phlebography revealed normal function of the proximal femoral valves in 92 % of streptokinase treated high thromboses compared with 13 % of those treated with heparin These phlebographic results were considered to be a valid prognostic indicator of the eventual development of the postthrombotic syndrome Allergic reactions were seen in 39 % and minor haemorrhagic complications in 18 % of the streptokinase treated cases The therapeutic benefit of streptokinase therapy in this study was found to outweigh any disadvantages incurred by observed complications

Key words thromboembolism streptokinase heparin
Acta Med Scand 205 293 1979

Deep vein thrombosis is a potentially serious condition giving rise to both early complications in the form of thromboembolism and to late complications characterized by the postthrombotic syndrome with associated varicose eczema and gravitational ulceration The antithrombotic effect of heparin and the thrombolytic effect of streptokinase are well documented in this condition (1 2 4 6 7 8 19 28) In previous comparative studies streptokinase has been consistently found to be more effective than heparin in achieving both complete and partial thrombolysis in acute and subacute thrombosis (9 14 23 29) A reduced incidence of the postthrombotic syndrome as a consequence of streptokinase treatment remains however to be conclusively demonstrated

In the present study a comparison between the results of streptokinase and heparin treatment in acute deep vein thrombosis is presented and correlated to posttherapy high valvular venous function as assessed by retrograde phlebography

PATIENTS AND METHODS

Before the start of the study standardization criteria were drawn up for the allocation of patients into the streptokinase or heparin treatment regime groups Only patients under the age of 70 years having a high thrombosis i e thrombosis in or proximal to the femoral vein and with a clinical age of the thrombosis of less than 7 days were accepted for the study On admission all cases were investigated for possible coagulopathy as well as chronic hypercoagulable states Thus cases of malignancy hepatic or renal failure disseminated intravasal coagulation or secondary fibrinolytic states were excluded On the same grounds patients who had undergone recent surgery or invasive diagnostic procedures were not included in the study

During the period June 1973–May 1977 35 patients were included in the study All were initially considered for thrombolytic therapy but only 18 finally received streptokinase treatment The remaining 17 were treated with heparin Of these one patient had received streptokinase treatment 3 years earlier complicated by a prolonged fall in blood pressure (BP) and as a consequence heparin treatment was chosen Two patients with moderate hypertension one patient with a mild thrombocytopenia and hypogonadismus testicularis and one with a history of three minor cerebral strokes were considered to exhibit contraindications for streptokinase but not for heparin treatment In three cases streptokinase treatment was started but discontinued during the first treatment hours because of allergic reactions in one case fall in BP in the second and haematuria probably due to renal stones in the third Thereafter they were treated with heparin Finally 9 cases considered to

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Abbreviations APTT=activated partial thromboplastin time FDP=fibrin/fibrinogen degradation products BP=blood pressure

Table I *Extension and localization of thrombosis assessed by antegrade phlebography*

Thrombotic involvement	Heparin group (n=17)	Streptokinase group (n=18)	Total (n=35)
Crural veins + v femoralis	13	14	27
Crural veins + v femoralis + v iliaca	3	3	6
V femoralis + v iliaca	1	1	2

be at risk if given streptokinase treatment because of technicalities involving ward personnel and available laboratory resources upon admission were treated with heparin

No previous symptoms of thrombotic disease or venous insufficiency in the affected leg were observed in 31 of the 35 patients One patient in the heparin group had experienced bilateral crural thrombosis with pulmonary emboli 36 years previously and one in the streptokinase group had been treated with oral anticoagulants for a suspect crural thrombosis 10 years before Neither of these patients demonstrated clinical signs of the postthrombotic syndrome One patient in each treatment group had exhibited symptoms of repeated superficial thrombophlebitis several years prior to the study but no clinical signs of deep vein involvement could be demonstrated Thus no clinical evidence of permanent venous valvular damage ...ing the present thrombotic episode could be noted ...ny patient in either treatment group

The phlebographic size and localization of the acute thromboses did not differ between the two treatment groups as shown in Table I The two groups are comparable also with regard to coexistent disease states in other organ systems and with regard to sex and age distribution the mean age of the streptokinase group is 59 2 years (S D 7 9) and of the heparin group 54 8 years (S D 15 8)

Phlebographic and plethysmographic examinations

After initial clinical assessment all patients underwent ascending phlebography according to Greitz (12) to determine the localization and extent of the thrombosis and to eliminate signs of previous thrombotic occlusion Patients in the streptokinase group underwent phlebography one or more times during the course of thrombolytic therapy Venous plethysmography with determinations of venous emptying times was performed upon admission after 10 days and 2–3 months after therapy The value of this non invasive method in the diagnosis of deep vein thrombosis and in assessing the effect of therapy has been described previously (3 13) Follow up ascending phlebography was performed 1–2 months after treatment to establish the objective results of thrombolytic and heparin therapy High valvular venous function was studied by retrograde phlebography in 20 cases

Laboratory investigations

Hb leukocyte count serum creatinine urine analysis for protein glucose and cells liver function tests—including bilirubin ASAT ALAT ALP and serum protein electrophoresis—and blood group determinations were performed in all patients Coagulation screening parameters were conducted on admission and included determinations of the bleeding time using the Ivy method according to Nilsson et al (18) modified by the use of an apparatus constructed by the Department of Medical Technology Karolinska Sjukhuset Stockholm Sweden (normal value 5–7 min) and a platelet count (16) (reference value 150–365 10^9/l) to assess primary haemostasis Screening of chemical haemostasis included determinations of activated partial thromboplastin time (APTT) (24) using orthodiagnostics reagents (normal value <45 sec) and normotest and thrombotest (19 20) (reference values normal >65% therapeutic range 5–51% (Nyegaard)) Heparin treatment was monitored by repeated APTT determinations Thrombin clottable fibrinogen determinations (27) (normal value 1 4–3 0 g/l) thrombin times (17) (normal value 20 sec) (Pentapharm) and quantitation of fibrin/fibrinogen degradation products (FDP) with the latex method (Wellcotest) (22) (normal values 0–10 mg/l) were performed at least once daily during streptokinase treatment The Wellcotest latex method was chosen due to its specificity for the end D and E fragments occurring in the complete thrombolytic degradation of fibrinogen The initial dose of streptokinase was titrated by a modification of Fischbacher s method (11) In some patients especially those with high levels of FDP reptilase times were also performed (Pentapharm) (17) (normal values 20 sec) however the use of reptilase for fibrinogen determinations was not found necessary since quantitation could be satisfactorily achieved using thrombin

Treatment regimes

Heparin group All patients recieved 45 000 IU heparin daily either as intermittent therapy in four equally divided doses or as a continuous infusion under APTT control once or twice daily Heparin dosage was considered adequate when APTT values remained constant between 60 and 120 sec during therapy Dicoumarol or warfarin sodium treatment was commenced at the same time as heparin therapy which in general could be discontinued after 5 days or when a thrombotest value of 5–15% was obtained Subsequent treatment with oral anticoagulants was continued for 3–6 months with thrombotest determinations every 1–3 weeks

Streptokinase group In the majority of patients thrombolytic therapy was conducted in the Intensive Care Unit Special attention was drawn to the identification and bandageing of possible bleeding sources from previous injection sites Corticosteroids in the form of 100 mg hydrocortisone phosphate were given i v *before* streptokinase therapy and peroral prednisolone 5–10 mg t d s during the course of the treatment Streptokinase (Kabikinas®) dissolved in 5 5% glucose was administered via an infusion pump At the start of the study streptokinase was given in an initial dose of 250 000 IU in 100 ml 5 5% glucose in 30 min and thereafter as a

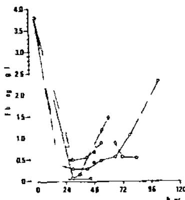


Fig 1 Effect of streptokinase on fibrinogen level O=4 patients in whom the thrombolytic effect has been followed up continuously ●=spot test during treatment in 9 patients

maintenance dose of 100000 IU per hour In the latter part of the investigation comprising by far the majority of cases laboratory resources were available to titrate the initial dose required in each individual case and to follow the thrombolytic effect by determination of thrombin time thrombin clottable FDP Criteria for adequate thrombolytic effect were considered met when fibrinogen levels less than 0 8 g/l were obtained the thrombin time was maintained between 2–4 times the normal value and when FDP levels remained high during treatment Streptokinase therapy was maintained for 3–4 days and terminated when an overall assessment of the clinical effect an ascending phlebography and coagulation parameters indicated that no further lytic effect could be expected Two hours after discontinuation of streptokinase treatment heparin and oral anticoagulant therapy was started as described in the heparin group Treatment with oral anticoagulants was continued for 3–6 months Similar dosages of warfarin sodium were used in both groups and no patient showed increased or decreased tolerance to anticoagulation throughout the treatment period of 3–6 months

During the study period none of the patients in either treatment group received medication containing acetylsalicylic acid or other platelet-inhibiting substances

The statistical analysis was performed using significance limits for the fourfold table test

RESULTS

The overall grade of thrombolysis was determined by a comparative study of phlebographic findings before therapy and at the follow up examination 1–2 months later In addition in 20 of the 35 cases studied high venous valvular function was investigated by retrograde phlebography

During streptokinase and heparin treatment biochemical tests of renal and hepatic function remained unaltered Acute reactive changes and leucocytosis were seen in streptokinase treated patients who developed manifest allergic reactions During the first 18–24 hours of streptokinase therapy marked prolongations of the APTT and thrombin times were observed but normalization of the APTT was achieved subsequently The effect of streptokinase on fibrinogen level is shown in Fig 1 Very high levels of FDP (>640 mg/l) were demonstrated initially in all streptokinase patients but in those 6 cases resulting in poor or no thrombolysis reduced FDP levels (<80 mg/l) were seen as early as on the second treatment day However in cases in whom complete lysis was attained this FDP reduction became evident during the 3rd–4th treatment day The daily FDP screening procedure was considered of value in the rough estimation of the degree of lysis during ensuing therapy and in connection *with clinical data it was found to be a use*ful indicator for discontinuation of thrombolytic therapy

The immediate therapeutic results of streptokinase and heparin are presented in Table II Complete lysis was evident in one (6%) of the 17 patients in the heparin group and in 8 (44%) of the 18 patients in the streptokinase group This difference is statistically significant ($p<0.05$) It is noteworthy that the majority of cases of complete lysis in the streptokinase group were observed when the clinical history of the thrombosis was shorter than 3 days

The findings at retrograde phlebography performed at the 1–2 month follow up investigation are shown in Table III High venous valvular insufficiency was seen in 7 of 8 patients treated with heparin In contrast 11 of 12 patients in the

Table II *Degree of posttherapeutic thrombolysis as assessed by antegrade phlebography*

A=complete B=partial C none or minimal thrombolysis

Age of thrombosis (d)	Heparin group ($n=17$)			Streptokinase group ($n=18$)		
	A	B	C	A	B	C
0–3	1	4	3	6	3	3
4–7	0	1	8	2	1	3
Total	1	5	11	8	4	6

Table III *Posttherapeutic high venous valvular function as assessed by retrograde phlebography in 20 patients*

A=complete B=partial C=none or minimal thrombolysis

	Heparin group (*n*=8)			Streptokinase group (*n*=12)		
	A	B	C	A		C
Normal proximal femoral valve	1			8	2	1
Damaged proximal femoral valve		4	3			1

streptokinase group were found to have normal high venous valvular function

Side-effects and complications of streptokinase and heparin therapy are shown in Table IV Therapy was rapidly discontinued in 3 patients receiving streptokinase because of hyperpyrexia and rigors fall in BP and haematuria probably due to renal stones In the streptokinase group fever between 38 and 39°C was a common finding despite corticosteroid therapy In seven cases in the streptokinase group allergic reactions in the form of urticaria Quincke s oedema or serum sickness were observed The two cases of serum sickness occurred 3–4 days after the completion of the ase treatment while the majority of the r allergic manifestations occurred during ensu therapy These reactions were not of such severity that discontinuation of the streptokinase therapy became necessary Small haemorrhages in the form of petechiae ecchymoses and bleeding or haematomas from injection sites occurred to the same extent in the two groups No significant haemorrhages with hypovolaemia were seen during either streptokinase or heparin therapy No definite difference in the incidence of pulmonary embolism was evident during the course of treatment in the two groups

DISCUSSION

High deep vein thrombosis is predominantly found to give rise to valvular insufficiency and post thrombotic syndrome sequelae (5 15) The present study was designed to compare the results of treatment of high thrombosis with special reference to posttherapeutic valvular function using two regimes with different modes of action Streptokinase through its rapid thrombolytic action could be expected to prevent permanent valve damage (9 13) Heparin on the other hand clinically terminates propagation of thrombi through its anticoagulant effects but since thrombolysis is achieved by the slower endogenous fibrinolytic mechanisms a higher risk for valvular damage would follow

In the streptokinase group 66% of cases of acute thrombosis had undergone complete or partial lysis and in this respect the results are consistent with previous documentation (9 10 25 26) It should be emphasized however that the 4 cases (22%) with partial lysis must be considered probable therapeutic failures with regard to preservation of valvular function On the other hand therapeutic success with complete thrombolysis was achieved in 8 (44%) of 18 cases In 6 of these 8 patients the clinical history was less than 3 days These results confirm and emphasize the significance of the clinical age of the thrombosis in the therapeutic outcome and the importance of rapidly instituted and adequate thrombolytic treatment

In the heparin group the frequency of complete and partial thrombolysis was 35% constituting a high percentage in comparison with previous investigations (10 13 14 23) This discrepancy can be explained on the basis of variations in the time of the follow up phlebography since this examination performed two months after therapy may include cases of late spontaneous partial lysis and completed or ensuing recanalization in addition to the initial lysis Complete early spontaneous lysis however was demonstrated in only one (6%) of 17 cases against 44% in the streptokinase group

Retrograde phlebography 1–2 months after streptokinase therapy demonstrated normal proximal femoral valvular function in 11 of 12 cases treated for high thrombosis This result indicates that early total thrombolysis in these cases may

Table IV *Complications during treatment*

	Heparin group (*n*=17)	Streptokinase group (*n*=18)
Fever of 38–40 C	0	16
Allergic reaction	0	7
Minor bleeding	2	3
Significant haemorrhage	0	0
Pulmonary embolism	1	1

have prevented permanent damage to the high valvular system In the heparin group however 7 out of 8 cases of which 4 showed partial thrombolysis had radiological signs of high venous valvular insufficiency indicating that partial thrombolysis is not necessarily accompanied by a corresponding decrease in the frequency of high valvular lesions Only one patient with complete early thrombolysis showed normal high venous function For various reasons mainly patient hesitation for invasive follow up investigation 15 patients (9 in the heparin and 6 in the streptokinase group) were not studied by retrograde phlebography Since no obvious difference in thrombolytic effect as assessed by post therapeutic antegrade phlebography was demonstrated in these 15 patients it is conceivable that the 20 patients in whom retrograde phlebography was performed are representative for each treatment regime

In previous investigations minor bleeding during streptokinase therapy has been estimated to occur within the frequency range of 25–65% major haemorrhage usually resulting in discontinuation of therapy in 15–30% of cases (10 14 23) In the present study haemorrhagic side effects were much less frequent with minor haemorrhages in 12% of cases in the heparin group and 17% in the streptokinase group No major haemorrhages occurred On the other hand allergic reactions were more prominent and such manifestations were seen in 7 (39%) of the 18 patients treated with streptokinase Pulmonary embolism during treatment was seen in one of the 18 patients in the streptokinase group and in one of the 17 patients in the heparin group

CONCLUSION

In the present study streptokinase was found to be more effective than heparin in the lysis of high thrombosis with a clinical history of less than seven days Preservation of high valvular venous function as assessed by retrograde phlebography was clearly evident in the streptokinase group as opposed to the heparin group Side effects and complications of streptokinase therapy were of minor degree when assessed in relation to the therapeutic results The low frequency of bleeding complications reported was considered to be due to strict adherence to the criteria drawn up for patient selection the quality of patient care and observation maintained in the Intensive Care Unit and to availability of laboratory parameters in monitoring therapy

The preservation of normal high valvular function after the streptokinase treatment regime was considered a prognostic indicator that a markedly reduced incidence of the postthrombotic syndrome could be expected in this patient group A five year follow up with special interest in the emergence of the postthrombotic syndrome in this patient series is in progress

REFERENCES

1 Adar R & Salzman E W Treatment of thrombosis of veins of the lower extremities N Engl J Med 292 348 1975

2 Aggeler P M & Kosmin M Anticoagulant prophylaxis and treatment of venous thrombo-embolic disease In Thrombosis (ed S Sherry K M Brinkhous E Genton et al) pp 639–689 National Academy of Sciences Washington D C 1969

3 Ahlback S Bygdeman S & Watz R The value of venous plethysmography in the diagnosis of venous thrombosis and for evaluation of the therapeutic results Standardization of Cardio Angiological Methods 4 1977

4 Bauer G Clinical experiences of a surgeon in the use of heparin Am J Cardiol 14 29 1964

5 Bieger R Boekhout Mussert R J Hohmann F & Loeliger E A Is streptokinase useful in the treatment of deep vein thrombosis? Acta Med Scand 199 81 1976

6 Brogden R N Speight T M & Avery G S Streptokinas a review of its pharmacology mechanism of action and therapeutic uses Drugs 5 357 1973

7 Browse N L Lea Thomas M & Pim H P Streptokinase in deep vein thrombosis Br Med J 3 717 1968

8 Chavatzas D & Martin P A study of streptokinas in deep vein thrombosis of the lower extremities Vasa 4 68 1975

9 Common H H Seamon A J Rosch J Porter J M & Dotter Ch Deep vein thrombosis treated with streptokinase or heparin Angiology 27 645 1976

10 Duckert F Muller G Nyman D Benz A Prisender S Madar G da Silva M A Widmer L K & Schmitt H E Treatment of deep vein thrombosis with streptokinase Br Med J 1 479 1975

11 Fischbacher W Beitrag zur fibrinolytischen Therapie mit Streptokinase und Fibrinolysin Thromb Diath Haemorrh 6 457 1961

12 Greitz T The technique of ascending phlebography of the lower extremity Acta Radiol 42 421 1954

13 Johansson E Ericson K & Zetterquist S Streptokinase treatment of deep venous thrombosis of the lower extremity Clinical phlebographic and plethysmographic evaluation of early and late results Acta Med Scand 199 89 1976

14 Kakkar V V Flanc C Howe C T O Shea M &

Flute P T Treatment of deep vein thrombosis A trial of heparin streptokinase and arvin Br Med J 1 806 1969
15 Kakkar V V Howe C T Laws J W & Flanc C Late results of treatment of deep vein thrombosis Br Med J 1 810 1969
16 Kristensson A Zur Methodik der Thrombozyten zahlung beim Menschen Acta Med Scand 57 301 1928
17 Latallo Z S Wegrzynowicz Z Teisseyre E & Kopec M Simple and rapid evaluation of the intravascular coagulation and fibrinolytic states by application of protamine sulphate and Reptilase R Scand J Haematol (Suppl) 13 261 1971
18 Nilsson I M Magnusson S & Borchgrevink C The Duke test and Ivy methods for determination of the bleeding time Thromb Diath Haemorrh 10 223 1963
19 Olow B Johanson C Andersson J & Eklof B Deep venous thrombosis treated with a standard dosage of streptokinase Acta Chir Scand 136 181 1970
20 Owren P A A new method for controlling anticoagulant therapy Lancet 2 754 1959
21 Owren P A & Strandli O K Normotest Farmakoterapi 1 14 1969
22 Pitcher P M Preparation of a rapid slide test for the detection of fibrinogen degeneration products (FDP) in serum and a preliminary report of its use as a chemical screening test 2nd Congress Int Soc on Thromb and Haemostasis Abstract 282 Oslo 1971
23 Porter J M Seaman A J Common H H Rosch J Eidemiller L R & Calhoun A D Comparison of heparin and streptokinas in the treatment of venous thrombosis Am Surg 41 511 1975
24 Proctor R R & Rapaport S I The partial thromboplastin time with kaolin Am J Clin Pathol 36 212 1961
25 Robertson B R On thrombosis thrombolysis and fibrinolysis Acta Chir Scand (Suppl) 421 51 1971
26 Tsapogas M J Peabody R A Wu K T Karmody A M Devaraj K T & Eckert C Controlled study of thrombolytic therapy in deep vein thrombosis Surgery 74 973 1973
27 Vermylen C de Vreker R A & Verstraete M A rapid enzymatic method for the assay of fibrinogen fibrin polymerisation time (FPT test) Clin Chim Acta 8 418 1963
28 Verstraete M The present status of thrombolytic agents Drugs 5 353 1973
29 Warner W L Frazer H & Maloy J Results of controlled clinical trials of streptokinase in venous thrombosis Int J Clin Pharmacol 10 154 1974

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Influence of High Plasma Concentrations of Free Fatty Acids on Heart Rhythm in Healthy Fasting Men

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ABSTRACT *Ten healthy male students in regular* sinus rhythm fasted for 66 hours Their overnight fasting plasma concentration of free fatty acids (FFA) was 455±104 μmol/l (mean ± S E M , $n=7$), the reference value of our laboratory, measured in another normal population of young men, being 344±28 μmol/l ($n=10$) After 42 and 66 hours of fasting, the plasma concentration of FFA rose to 1198±181 ($p<0\ 01$ $n=10$) and 1471±89 μmol/l ($p<0\ 001$, $n=10$) respectively During the last 24 hours of fasting, the heart rhythm was monitored continuously by means of a Holter recorder and computer No arrhythmias were observed indicating that elevated plasma concentrations of FFA, exceeding those reported in patients with acute myocardial infarction are well tolerated by the healthy human myocardium

Key words FFA heart rhythm

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Both experimental (8) and clinical (2 10 12) observations have indicated an association between high plasma concentrations of free fatty acids (FFA) and serious ventricular arrhythmias in ischemic heart disease Possible mechanisms for an arrhythmogenic effect of FFA include an increased myocardial oxygen demand (9) and a detergent effect on the cell membrane (6) Evidence has also been provided that a high FFA/albumin molar ratio in the perfusion medium may produce arrhythmias even in the normal non ischemic rat heart (15)

In the present study we therefore examined the ability of high FFA levels to provoke arrhythmias even in the absence of myocardial ischemia in man Raised plasma concentrations of FFA were obtained by 66 hours of fasting

SUBJECTS AND METHODS

Ten male students aged 21–26 years were included in the study None had a history of palpitation or of any significant disease Before entering the study they underwent a complete physical examination Hb ESR serum creatinine and resting ECG were normal in all The subjects fasted for 66 hours with free intake of water They undertook normal activities but avoided heavy physical exercise *Smoking was not permitted*

Every morning i e at 42 and 66 hours after the initiation of fasting arterial blood pressure heart rate and plasma concentrations of FFA were measured The estimation of FFA concentration after overnight fasting was separated in time from the fasting procedure in order to record the real basic levels in these subjects FFA was assayed by means of the titrimetric method of Dole (1) as modified by Trout et al (14) The normal overnight fasting value in our laboratory calculated on the basis of 10 other normal subjects values is 344±28 μmol/l

During the last 24 hours of fasting a two-lead ECG was recorded continuously by means of an Avionic Holter recorder model 425 using conventional electrode positions The recordings were analyzed by means of an Avionic electroscanner model 660 In our experience simultaneous computer and visual interpretation of heart rhythm with repeating display of the tape is a highly reliable method for quantitating ventricular premature beats Since practically no arrhythmias were present during the last 24 hours we found that basal 24-hour recordings were not essential

The *t* test was used for statistical comparisons

RESULTS

The fasting procedure was well tolerated by all subjects and compliance with the regimen was rigid Plasma FFA concentrations showed a wide variation but rose considerably in all subjects during

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Abbreviations FFA=free fatty acids ECG=electrocardiogram AMI=acute myocardial infarction

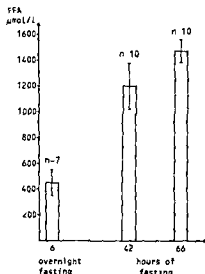


Fig 1 Effect on plasma FFA during 66 hours of fasting in 10 healthy men

fasting (Fig 1) The mean value after overnight fasting was 450±104 µmol/l (mean ± S E M , n=7) after 42 hours 1 198±181 µmol/l and after 66 hours 1471±89 µmol/l The values recorded at 42 and 66 hours differ highly significantly both from the basic values of the subjects studied and from the normal value indicated above (p<0 001)

All ECGs were of good technical quality In 8 jects no arrhythmias were recorded One had a isolated ventricular premature beats at rest be ore the study which disappeared during exercise he had no arrhythmias during the 24 hour recording Another subject had 2 ventricular premature beats during the 24 hours

DISCUSSION

The fasting procedure applied in this study induced on average a threefold increment of serum FFA levels The elevation was consistent and stable in all subjects throughout the period of ECG recording The levels observed exceed those associated with a substantial increase in the incidence of arrhythmias during acute myocardial infarction (AMI) (5, 7 10) Thus Oliver et al (10) found that 25% of their 200 patients with AMI had a maximum FFA level exceeding 1 200 µEq/l Even though this level lasted for only a few hours in this patient group 94% had serious arrhythmias during hospitalization

Other workers in the field have questioned the association between elevated FFA concentrations and arrhythmias even in patients with AMI (4 11, 13) However most data substantiate the view that there is not only an association but also a causal relationship between FFA and arrhythmias in this disease (2 10 12)

As mentioned, in spite of the elevated FFA values no induction of arrhythmias was observed in our study This finding strongly indicates that high FFA levels alone are not capable of provoking arrhythmias in the healthy human myocardium This conclusion is apparently at variance with the observation that a high concentration of FFA may provoke arrhythmias in the isolated non ischemic rat heart (15) However the latter observation may be explained by the use of unphysiologically high FFA/albumin molar ratios in the perfusate by the rat heart being already borderline ischemic or by species differences

An ability of FFA to provoke arrhythmias in the diseased but not in the healthy myocardium is in good accordance with present knowledge of myocardial metabolism High plasma concentrations of FFA are known to increase the myocardial oxygen consumption (9) In the normal heart with a normal vascular bed and an intact autoregulation this increased consumption is easily compensated for by an increased blood flow (3) and no regions of the heart are thus rendered hypoxic In the diseased heart however increased oxygen requirements may be able to provoke focal ischemia and hence arrhythmias (16)

REFERENCES

1 Dole E P A relation between non-esterified fatty acids in plasma and the metabolism of glucose J Clin Invest 35 150 1956
2 Gupta D K Jewitt D E Young R Herzog M & Opie L H Increased plasma free fatty acid concentrations and their significance in patients with acute myocardial infarction Lancet 2 1209 1969
3 Haddy F J Physiology and pharmacology of the coronary circulation and myocardium particularly in relation to coronary artery disease Am J Med 47 274 1969
4 Hagenfeldt L & Wester P O Plasma levels of individual free fatty acids in patients with acute myocardial infarction Acta Med Scand 194 357 1973
5 Kurien V A & Oliver M F Serum free fatty acids after acute myocardial infarction and cerebral vascular occlusion Lancet 2 122 1966
6 — A metabolic cause for arrhythmias during acute myocardial hypoxia Lancet 1 813 1970

7 — Free fatty acids during acute myocardial infarction Prog Cardiovasc Dis 13 361 1971
8 Kurien V A Yates P A & Oliver M F The role of free fatty acids in the production of ventricular arrhythmias after acute coronary artery occlusion Eur J Clin Invest 1 225 1971
9 Mjøs O D Effect of free fatty acids on myocardial function and oxygen consumption in intact dogs J Clin Invest 50 1386 1971
10 Oliver M F Kurien V A & Greenwood T W Relation between serum free fatty acids and arrhythmias and death after acute myocardial infarction Lancet 1 710 1968
11 Opie L H Norris R M Thomas M Holland A J Owen P & Van Noorden S Failure of high concentrations of circulating free fatty acids to provoke arrhythmias in experimental myocardial infarction Lancet 1 818 1971
12 Rowe M J Neilson J M M & Oliver M F Control of ventricular arrhythmias during myocardial infarction by antilipolytic treatment using a nicotinic acid analogue Lancet 1 295 1975
13 Rutenberg H L Pamintuan J C & Soloff L A Serum free fatty acids and their relation to complications after acute myocardial infarction Lancet 2 559 1969
14 Trout D L Estes E H Jr & Friedberg S J Titration of free fatty acids of plasma a study of current methods and a new modification J Lipid Res 1 199 1960
15 Willerbrands A F ter Welle H F & Tasseron S J A The effect of a high molar FFA/albumin ratio in the perfusion medium on rhythm and contractility of the isolated rat heart J Mol Cell Cardiol 5 259 1973
16 Wit A L & Friedman P L Basis for ventricular arrhythmias accompanying myocardial infarction Arch Intern Med 135 459 1975

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Citrate in Plasma and Urine During Total Fasting

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ABSTRACT Plasma citrate was determined in 12 obese subjects who underwent total fasting for 10 days Mean plasma citrate concentration rose significantly from 128 before to 205 μmol/l on the 10th day of fasting Plasma citrate rose continuously during fasting in seven subjects in whom daily determinations were carried out The 24-hour urinary citrate excretion was followed in six subjects A significant decrease was found from 2 91 mmol/24 h in the prefasting state to 0 25 mmol/24 h at the end of the fast Intravenous glucose tolerance tests were performed before and on the 10th day of fasting K_{IVGTT} decreased significantly and was inversely related to plasma citrate concentration on the 10th day of fasting The results agree well with the concept that an increased citrate level of tissues is of regulatory importance for the decreased glucose utilization during fasting in man

Key words plasma citrate urinary citrate fasting glucose tolerance obesity

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Metabolic adaptations to fasting include decreased glucose utilization increased gluconeogenesis and fat oxidation Alterations in levels of glucoregulatory hormones and in interorgan substrate fluxes have been studied extensively in man (2) However little is known about the control mechanisms by which tissues adapt oxidation of fuels to changes in substrate supply during fasting in man

Years ago Randle et al (15) provided evidence that the decreased glucose utilization of rat heart during conditions with increased fat oxidation (starvation diabetes) could be explained by an intracellular accumulation of citrate due to an inhibitory effect of citrate on the key glycolytic enzyme phosphofructokinase (PFK) (14) Attempts to demonstrate this control mechanism in other tissues especially the large mass of skeletal muscle have for a long time failed (18) Recently increased availability of fatty acids has been demonstrated to increase citrate concentration of skeletal muscle in rat and to inhibit glucose phosphorylation at the site of PFK simultaneously (16 17) We have demonstrated arteriovenous plasma citrate differences across the leg and the splanchnic vascular bed in man during exercise which vary in agreement with the concept that a citrate inhibition of PFK is of regulatory importance for glucose utilization of muscle as well as for hepatic glycolysis and gluconeogenesis (22) Thus growing evidence had appeared that a citrate inhibition of glucose phosphorylation may be of importance for the overall glucose utilization

The present study was undertaken to examine whether changes in citrate levels in plasma and urine could be demonstrated during fasting in man and if so whether they could be related to the decrease in glucose utilization evaluated by changes in glucose tolerance

SUBJECTS PROCEDURES AND METHODS

The study included 12 obese subjects nine women and three men aged 18–58 years (mean 31) with a body weight of 137–235% (mean 189) of ideal weight (4) All were otherwise healthy and took no medicine On arrival at the department the subjects were given a common everyday diet for at least 3 days and then subjected to total fast for 10 days during which only water was given After fasting they were fed a 900 kcal diet for further treatment

Fasting was controlled by daily examinations for ketonuria (Acetest®) Plasma standard bicarbonate was followed at regular intervals

Intravenous glucose tolerance tests (IVGTT) were performed in the morning after an overnight fast before

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Abbreviations PFK=phosphofructokinase IVGTT=intravenous glucose tolerance test K_{IVGTT}=rate constant during IVGTT

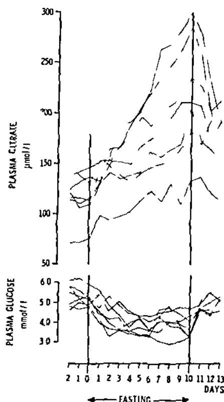


Fig 1 Concentrations of citrate and glucose in plasma in 7 obese subjects during 10 days of total fasting

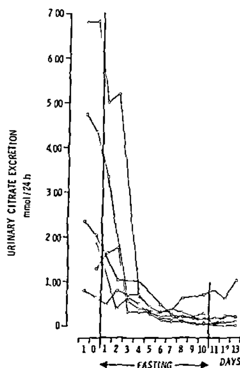


Fig 2 24 hour urinary citrate excretion in 6 obese subjects during 10 days of total fasting

starvation and on the 10th day of fasting Glucose 25 g was injected during 4 min in an antecubital vein and blood samples were taken from the contralateral vein at −30 15 0 5 10 20 30 40 50 60 75 and 90 min for determination of plasma citrate glucose and insulin

In seven subjects concentrations of citrate and glucose in plasma were determined daily at 8 00 a m (Fig 1) The 24-hour urinary citrate excretion was determined in six of these seven subjects (Fig 2)

Analytical methods The concentrations of citrate in plasma and urine were determined by a method using citrate lyase (21) Plasma glucose was measured using an o-toluidine method (5) Plasma insulin was analysed by the alcohol precipitation method of Heding (6) and plasma standard bicarbonate by a routine method

Calculations and statistics Rate constants (K_{IVGTT}) of plasma glucose decay during IVGTT were calculated as ln 2/$T_{\frac{1}{2}}$ (10) Wilcoxon s test for pair differences was employed for paired observations Non randomness of changes in plasma citrate during IVGTT was examined by Friedman s method for randomized blocks (20) Spearman s Rho (*R*) was used in correlation studies

RESULTS

Weight loss during fasting averaged 8 1 kg (range 6 4–9 0) Ketonuria was found in all subjects after 1–4 days fasting Plasma standard bicarbonate decreased significantly from 24 5 mmol/l (range

Table I *Plasma citrate glucose insulin and K_{IVGTT} in 12 obese subjects*
Mean of three determinations on the day of IVGTT range is given in parentheses

	Citrate (μmol/l)	Glucose (mmol/l)	Insulin (μU/ml)	K_{IVGTT} (10^{-2}×min^{-1})
Before fasting	128 (79–165)	5 0 (4 7–5 7)	18 (1–26)	1 30 (0 64–2 24)
10th day of fasting	205 (131–284)	3 9 (3 2–4 7)	7 (0–13)	1 00 (0 52–1 47)
p	<0 01	<0 01	<0 01	<0 05

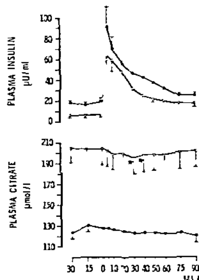


Fig 3 Plasma insulin and citrate concentrations during IVGTT in prefasting state (■—■) and on the 10th day of fasting (□—□) ($n=12$) (mean ± S.E.M.) ★=Concentrations significantly lower than before glucose injection ($p<0.05$)

26.9–22.6) before fasting to 19.8 mmol/l (range 16.6–25.4) on the 10th day of fasting ($p<0.01$)

Mean values of plasma citrate, glucose and insulin concentrations and of K_{IVGTT} in prefasting state and on the 10th day of fasting are given in Table I. Plasma citrate concentrations had increased in all subjects to values on the 10th day of fasting from 128 to 245% of prefasting levels.

Fig. 1 gives daily plasma citrate concentrations in seven subjects. During fasting, citrate concentrations increased continuously in all. This contrasted to plasma glucose, which decreased to constant levels after 4–5 days. Plasma citrate concentrations returned towards prefasting levels in six of the subjects during 2–3 days' refeeding.

Fig. 2 shows the 24-hour urinary citrate excretion in 6 subjects. Citrate excretion varied from 0.79 to 6.82 mmol/24 h (mean 2.91) before fasting. During fasting, citrate excretion decreased and was constantly low after 4–5 days. Mean urinary citrate excretion after 10 days' fasting was 0.25 mmol/24 h (range 0.05–0.72), significantly lower than in prefasting state ($p<0.05$).

Mean plasma citrate levels during IVGTT are given in Fig. 3. Plasma citrate did not change after glucose injection in prefasting state (non randomness $p>0.1$), while a small decrease of 6% of the concentration before injection was found 30 and 40 min after glucose administration on the 10th day of fasting (non randomness $p<0.025$, pair test $p<0.05$). Mean insulin response after glucose injection was smaller during fasting than before, though the difference was not significant at any time.

K_{IVGTT} decreased significantly during the fast (Table I). A significant negative correlation was demonstrated between plasma citrate concentration and K_{IVGTT} on the 10th day of fasting ($R=-0.76$, $p<0.01$). No such correlation could be demonstrated in the prefasting state but the same tendency was found ($R=-0.34$, $p>0.1$) (Fig. 4).

Plasma citrate concentration before and on the 10th day of fasting tended to be positively related to body weight (% of ideal weight in prefasting state) without correlating significantly ($R=0.30$, $p>0.1$ in prefasting state, $R=0.50$, $0.1>p>0.05$ on the 10th day of fasting).

DISCUSSION

The present findings, that plasma citrate increased progressively and that urinary citrate excretion was almost abolished, indicate an accumulation of citrate in the extracellular space during the 10 days of fasting. This can be due to a diminished removal from or an increased release of citrate to plasma water.

Liver (22) and kidney (13) are the sites of citrate uptake from plasma in postabsorptive man. Hepatic citrate uptake from plasma has been found to increase during fasting in dog (7). Studies from different laboratories revealed a constant fractional extraction, 20–30% of arterial plasma citrate concentration across kidney in man and dog in fed state (1, 8) and in the acidotic dog before and during i.v.

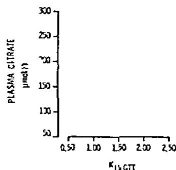


Fig. 4 Correlations between plasma citrate concentrations and K_{IVGTT} in prefasting state (○) ($R=-0.34$, $p>0.1$) and on the 10th day of fasting (●) ($R=-0.76$, $p<0.01$)

citrate administration (25) This was the case independently of whether urinary citrate excretion was abolished or within the range of mmol/h and during variations of plasma citrate concentration of <100->1 000 μmol/l Thus the abolished urinary citrate excretion cannot be held responsible for the increase in plasma citrate It is unlikely moreover that a diminished citrate uptake in liver and kidney tissues contributes to the rise in plasma citrate during fasting

There is evidence that an increased citrate output across skeletal muscle is responsible for the increase in plasma citrate during fasting We have demonstrated a release of citrate across resting human leg (22) Citrate output across hindquarter in the fasted dog increases after glucagon administration (7) Plasma glucagon increases during fasting in man (11)

During fasting glucose utilization of some tissues (skeletal muscle and kidney cortex in particular) is depressed and energy requirements are met from oxidation of fatty acids This adaptation is of vital importance for conserving available glucose for glucose requiring tissues especially the brain and blood cells (2)

Many reports have dealt with the question whether an increased oxidation of fatty acids is of causal importance for the decreased glucose utilization (impaired glucose tolerance) during fasting and conditions with increased plasma fatty acid level (19) In part an influence of inhibited fatty acid oxidation on glucose utilization is thought to be mediated through an increased citrate concentration of tissues due to the inhibitory effect of citrate on PFK the rate limiting enzyme of glycolysis (9) Citrate inhibits PFK from a variety of mammalian tissues including skeletal muscle heart (14) liver (24) kidney (23) and adipose tissue (3) Recently increased availability of free fatty acids in plasma has been demonstrated to increase the citrate concentration of muscles in resting rat and to inhibit glucose uptake and oxidation simultaneously (16 17) Our demonstration of an increased plasma citrate concentration during fasting which was inversely related to glucose tolerance supports the concept that an increased citrate concentration of tissues is of regulatory importance for the decreased glucose utilization during fasting in man

So far, an increased citrate concentration has been considered to be a local phenomenon of tissues The present finding of a marked increase in plasma citrate during fasting makes it possible that citrate in amounts of regulatory significance may be taken up by tissues In view of the above one may suggest a considerably citrate uptake in kidney after 10 days of fasting An increased citrate concentration inhibits glucose oxidation and increases gluconeogenesis in kidney cortex slides (12) There is no evidence that a citrate inhibition of PFK in the liver is of regulatory importance for hepatic gluconeogenesis during fasting (12)

In conclusion our finding of an increased plasma citrate level and of a decreased urinary citrate excretion agree well with the concept that an increased citrate level of tissues is of regulatory importance for the decreased glucose utilization during fasting in man

ACKNOWLEDGEMENTS

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REFERENCES

1 Brodwall E K & Laake H The renal metabolism of citric acid Acta Med Scand 174 501 1963
2 Cahill G F Jr Starvation in man N Engl J Med 282 668 1970
3 Denton R M & Randle P J Citrate and the regulation of adipose tissue phosphofructokinase Biochem J 100 420 1966
4 Diem K & Lenter C Scientific tables 7th ed p 712 Geigy Basle 1970
5 Feteris W A A serum glucose method without protein precipitation Am J Med Technol 31 17 1965
6 Heding L G A simplified insulin radioimmunoassay method In Labelled proteins in tracer studies (ed L Donato) p 345 Euratom Brussels 1966
7 Henneman D H & Shoemaker W C Effect of glucagon and epinephrine on regional metabolism of glucose pyruvate lactate and citrate in normal conscious dogs Endocrinology 68 889 1961
8 Herndorn R F & Freeman S Renal citric acid utilization in the dog Am J Physiol 192 369 1958
9 Krebs H A The Pasteur effect and relations between respiration and fermentation Essays Biochem 8 1 1972
10 Lundbæk K Intravenous glucose tolerance as a tool in definition and diagnosis of diabetes mellitus Br Med J 1 1507 1962
11 Marliss E B Aoki T T Unger R H Soeldner J S & Cahill G F Jr Glucagon levels and metabolic effects in fasting man J Clin Invest 49 2256 1970
12 Newsholme E A & Start C Regulation in metabolism pp 247-292 Wiley London 1973

13 Nieth H & Schollmeyer P Substrate utilization of the human kidney Nature 209 1244 1966
14 Parmeggiani A & Bowman R H Regulation of phosphofructokinase activity by citrate in normal and diabetic muscle Biochem Biophys Res Commun 12 268 1963
15 Randle P J Garland P B Hales C N & Newsholme E A The glucose fatty acid cycle Lancet 1 785 1963
16 Rennie M J & Holloszy J O Inhibition of glucose uptake and glycogenolysis by availability of oleate in well-oxygenated perfused skeletal muscle Biochem J 168 161 1977
17 Rennie M J Winder W W & Holloszy J O A sparing effect of increased plasma fatty acids on muscle and liver glycogen content in exercising rat Biochem J 156 647 1976
18 Ruderman N B Goodman M N Berger M & Hagg S Effect of starvation on muscle glucose metabolism studies with the isolated perfused rat hindquarter Fed Proc 36 171 1977
19 Ruderman N B Toews C J & Shafrir E Role of free fatty acids in glucose homeostasis Arch Intern Med 123 299 1969
20 Sokal R R & Rholf F J Biometry p 396 Freeman San Francisco 1969
21 Toftegaard Nielsen T A method for enzymatic determination of citrate in serum and urine Scand J Clin Lab Invest 36 513 1976
22 Toftegaard Nielsen T & Thomsen P E B Leg and splanchnic arteriovenous differences of plasma citrate in exercising man J Appl Physiol In press 1978
23 Underwood A H & Newsholme E A Properties of phosphofructokinase from rat kidney cortex Biochem J 97 6P 1965
24 — Properties of phosphofructokinase from rat liver and their relation to the control of glycolysis and gluconeogenesis Biochem J 95 868 1965
25 Vinay P & Lemieux G Effet du citrate et de l acide citrique sur l ammoniogenese renale chez le chien Union Med Can 102 1491 1973

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The Removal of Exogenous Triglycerides in Haemorrhagic Hyperlipidaemia in Rabbits

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ABSTRACT Bleedings (15 ml/day/kg b wt) on two consecutive days caused a threefold increase in plasma triglycerides (TG) in rabbits Both in normal and in haemorrhagic rabbits the elimination of injected TGs (fat emulsion) was exponential, the fractional removal rate in the haemorrhagic group being slower than normal In order to rule out the possible effect of the TG pool in haemorrhagic rabbits and the possible changes in endogenous TGs during the test, the TGs were fractionated by the polyvinylpyrrolidone density gradient method Again, both in normal and in haemorrhagic rabbits the elimination of exogenous TGs was exponential the removal rate of haemorrhagic animals being retarded Accordingly, the changes in endogenous TGs were negligible The results are regarded as indicative of an elimination defect as one factor in the pathogenesis of haemorrhagic hyperlipidaemia

Key words triglycerides hyperlipidaemia haemorrhagic anaemia triglyceride removal fat emulsion triglyceride fractionation

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The primary cause of the hyperlipidaemia developing as a result of repeated bleedings seems to be the loss of red blood cells (9 10) In many respects however the pathogenesis of this phenomenon has been unclear (8) In a recent study (7) there was some evidence of increased triglyceride (TG) synthesis in haemorrhagic hyperlipidaemia At the same time the fractional removal rate of TGs in haemorrhagic rabbits was found to be decreased *but it was inversely related to the starting level of* the TGs with the exception of the most anaemic animals Therefore it was considered necessary to fractionate the TGs in order to get a more exact idea of the behaviour of exogenous TGs and thereby of a possible elimination defect in haemorrhagic hyperlipidaemia This report presents the results of these experiments

MATERIAL AND METHODS

Experimental animals bleedings and fat loading tests Albino rabbits were used as experimental animals Their treatment and other experimental procedure were as described earlier (7) The removal rate (λ) of both total and exogenous TGs was calculated by computer in each case on the basis of at least 6 TG determinations The TG dose (as 20% olive oil emulsion) was 0 5 g/kg b wt in all tests given as a single infusion

Chemical analyses The determination of TGs was performed according to Van Handel and Zilversmit (5)

Fractionation of triglycerides in polyvinylpyrrolidone (PVP) gradient tubes The exogenous fat particles were separated from the endogenous TGs in PVP gradient tubes (3) measuring 16 by 64 mm (4) The PVP (Plasdone General Aniline and Film Co New York) had an average molecular weight of 40 000

The blood samples for PVP gradient fractionation were taken into tubes cooled in ice and the procedure was started immediately after sampling A plasma sample of 0 8 ml was taken for fractionation and 80 mg of NaCl and 0 2 ml of PVP solution were added Of this mixture 0 8 ml were injected onto the bottom of the gradient tube using a fine needle The tubes were then kept at 37 C for approximately 16 hours In some experiments 0 4 ml of plasma was taken for fractionation and the additions were reduced proportionally

A fine needle was then used to draw a 3 ml fraction from the bottom of the tube This bottom fraction contained endogenous triglycerides The top fraction of 7 ml contained the exogenous TGs This division was based on preliminary tests The fat emulsion added in vitro formed a turbid layer on the surface in the gradient tube but it also extended in the form of a more tenuous haze opacity down to a deeper level consistent with the stated volume of 7 ml The TG determinations were made from the top and bottom fractions When various quantities of diluted fat emulsion were added to rabbit serum in vitro this had no effect on the TG values of the bottom fraction

When 0 8 ml of plasma had been taken for fractionation the TG recovery was 85–90% against 105–110% when 0 4 ml of plasma had been used The same plasma volume was taken throughout from each animal

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Abbreviations TG = triglyceride ΔTG_0 = increase in TG extrapolated to zero time PVP = polyvinylpyrrolidone

Table I *Results of fat loading tests concerning exogenous TGs on normal and haemorrhagic rabbits*

Rabbit no	Plasma triglycerides (mmol/l) Starting level	ΔTG_0	k (%/min)	r
Normal				
1	0 19	16 9	2 22	0 9913
2	0 21	7 13	1 55	0 9608[b]
3	0 10	4 23	1 55	0 9232[b]
4	0 22	8 42	4 44	0 9768
5	0 13	10 8	3 23	0 9549
6	0 24	5 14	2 89	0 9636
7	0 22	3 50	3 45	0 9726
8	0 15	3 10	2 23	0 9690
9	0 16	4 75	3 92	0 9705
10	0 18	2 01	2 51	0 9087[b]
11	0 11	6 94	3 24	0 9249[b]
Mean	0 17	6 63	2 84	
S D		4 28	0 93	
±S D range	0 13–0 22			
Haemorrhagic				
12	0 56	16 9	1 46	0 9576
13	1 58	10 7	1 17	0 9912
14	1 46	6 87	1 13	0 9629
15	0 54	3 24	0 61	0 9820
16	0 18	3 16	0 76	0 9867
17	0 47	6 63	0 95	0 9785
18	0 78	7 38	1 31	0 9842
19	0 50	4 77	1 70	0 9205[b]
20	0 24	5 93	2 58	0 9043[b]
21	0 32	2 76	2 59	0 9418
22	0 65	6 34	1 52	0 9451
	0 25	8 36	3 03	0 9852
	0 35	4 55	1 15	0 9292[b]
	0 43	3 21	3 30	0 9657[b]
Mean	0 49[a]	6 49	1 66	
S D		3 77	0 86	
± S D range	0 24–0 92			

[a] Logarithmic based
[b] Not less than six observations per exponential regression determination Regression statistically significant at a level of $p<0\ 01$ in these cases at a level of $p<0\ 001$ in all the others

RESULTS

Normal rabbits

Fat loading tests were carried out in 11 normal rabbits having an average body weight of 3 28±0 53 (S D) kg and a mean (logarithmic based ± S D range) plasma TG value of 0 17 (0 13–0 22) mmol/l The elimination of total TGs was exponential The r values indicating the fit with a linear logarithmic graph were statistically significant at a level of $p<0\ 001$ in all but one animal ($p<0\ 01$) The k value of total TGs averaged 2 93±1 07%/min There was no correlation between log TG and log k ($r=+0\ 110$) or between the increase in TG values extrapolated to zero time (ΔTG_0) and k ($r=-0\ 090$)

The average k value of the exogenous TGs was 2 84±0 93%/min (Table I), or almost the same as that of the total TGs Again the elimination was exponential No correlation was found between log TG and log k ($r=+0\ 216$) nor between ΔTG_0 and k ($r=+0\ 107$) or between body weight and k ($r=+0\ 284$)

The changes in the endogenous TGs (PVP gradient bottom fraction) were somewhat variable and rather small The highest increase in these values observed at 15–65 min after fat infusion averaged 0 26±0 30 mmol/l

Haemorrhagic rabbits

The group of 14 rabbits was bled 15 ml/kg b wt on two consecutive days The fat loading test was carried out one day after the second bleeding As a result of the bleedings the mean Hb decreased from 125±11 to 59±4 g/l The mean TG increased from 0 14 (0 10–0 19) to 0 23 (0 13–0 44) mmol/l after one day ($p<0\ 005$) and to 0 49 (0 26–0 92) mmol/l after two days ($p<0\ 001$) Body weight did not decrease as a result of the bleedings

As in normal rabbits the elimination of total TGs was exponential The corresponding r values were statistically significant at the levels of $p<0\ 001$ $p<0\ 01$ and $p<0\ 05$ in 11 two and one animal respectively The mean k value of the total TGs was 1 55±0 92%/min which is clearly lower than normal (2 93±1 07) ($p<0\ 01$) A negative correlation was established between log TG and log k ($r=-0\ 565$ $p<0\ 05$) There was no correlation between body weight and k ($r=-0\ 054$) nor between ΔTG_0 and k ($r=+0\ 115$)

Again the elimination of exogenous TGs was exponential with statistically significant r values ($p<0\ 001$ in 10 and $p<0\ 01$ in 4 animals) (Table I) The mean maximal k value was 1 66±0 86%/min which is approximately the same as for total TGs and significantly lower than the exogenous TG removal rate in normal rabbits (2 84±0 93%/min $p<0\ 01$) There was no correlation between the log TG starting level and log k ($r=-0\ 264$) Neither were ΔTG_0 and body weight correlated with k ($r=-0\ 115$ and $-0\ 068$ respectively)

The highest increase in the endogenous TGs noted in this group 15–65 min after the fat infusion averaged 0 40±0 29 mmol/l the difference between

haemorrhagic and normal rabbits (0 26±0 30 mmol/l) being insignificant ($p>0\ 05$)

Dependence of fractional removal rate on triglyceride level

In the normal rabbits there was no correlation between log TG and log λ In the haemorrhagic group however a negative correlation was found between these figures ($r=-0\ 565$ $p<0\ 05$) Combining these groups gives an idea of the relationship between TG level and λ value in their entire range of variation Then a negative correlation is revealed between log TG and log λ ($r=-0\ 692$ $p<0\ 001$) The correlation between the TG levels and λ values of the exogenous TGs is weaker ($r=-0\ 504$ $p<0\ 05$)

DISCUSSION

It has been reported earlier (7) that the removal of total TGs both in normal and in haemorrhagic rabbits is exponential at least up to a concentration of 9–11 mmol/l above the starting level It was then assumed that the level of endogenous TGs does not change substantially during the fat loading test The present series confirmed that the elimination curve of the exogenous TGs is exponential At the same time the changes in the endogenous TGs were small In fact the exponential type of kinetic behaviour is evidence against any appreciable recirculation of the administered TGs from the tissues back into the blood stream (2) In man however changes in the endogenous TGs during the fat loading test have been reported (4) The exponential or first order kinetics of the exogenous TGs in rabbit contrasts with the findings in dog (1) and in man (4) In the latter the elimination of TGs was at first maximal which is consistent with zero order kinetics and it was not replaced by first order kinetics until the second phase The concentration at which the change of kinetics took place in the dog was rather low i e about 1 mmol/l above basal level (determined from whole blood) So at least for TGs in the form of artificial fat emulsion the rabbit appears to have a more efficient elimination system than the dog or man

In a previous study (7) the fractional removal rate of (total) TGs was found to be decreased in haemorrhagic hyperlipidaemia As expected the λ value was however inversely correlated with the starting level of TGs (i e TG pool) except in the most anaemic rabbits So the decrease could not be regarded as an indication of a true elimination defect It is true that there are observations according to which the fractional removal rate of TGs is not directly dependent on the endogenous TG pool (6) In the present series the elimination of the exogenous TGs in haemorrhagic rabbits was clearly slower than in normal animals Hence a defect in the elimination of TGs seems to be a contributory factor in the pathogenesis of haemorrhagic hyperlipidaemia Besides this earlier results (7) have suggested an increase in TG synthesis in haemorrhagic hyperlipidaemia.

REFERENCES

1 Carlson L A & Hallberg D Studies on the elimination of exogenous lipids from the blood stream. The kinetics of the elimination of a fat emulsion and of chylomicrons in the dog after single injection Acta Physiol Scand 59 52 1963
2 Farquhar J W Gross R C Wagner R M & Reaven G M Validation of an incompletely coupled two-compartment nonrecycling catenary model for turnover of liver and plasma triglyceride in man J Lipid Res 6 119 1965
3 Gordis E Demonstration of two kinds of fat particles in alimentary lipaemia with polyvinylpyrrolidone gradient columns Proc Soc Exp Biol 110 657 1962
4 Hallberg D Studies on the elimination of exogenous lipids from the blood stream Determination and separation of the plasma triglycerides after single injection of a fat emulsion in man Acta Physiol Scand 62 407 1964
5 Van Handel E & Zilversmit D B Micromethod for the direct determination of serum triglycerides J Lab Clin Med 50 152 1957
6 Harris K L & Felts J M Kinetics of chylomicron triglyceride removal from plasma in rats The effect of diet Biochim Biophys Acta 316 268 1973
7 Kerttula Y Fat loading tests in haemorrhagic hyperlipidaemia in rabbits Eur J Clin Invest 8 233 1978
8 Louhija, A Metabolic studies on haemorrhagic lipaemia in the rat Ann Med Exp Biol Fenn (Suppl) 2 1965
9 Punsar S Hartel G Louhija A & Heinonen O P Studies on haemorrhagic lipemia in rats with particular references to the effect of hypophysectomy Acta Endocrinol 34 473 1960
10 Starup U Undersøgelser over experimentel hyperlipaemi Munksgaard Copenhagen 1937

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Changes in Amylase, Hepatic Enzymes and Bilirubin in Serum upon Initiation of Alcohol Abstinence

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ABSTRACT The serum levels of bilirubin aspartate aminotransferase (ASAT) γ-glutamyltransferase (GT) and pancreatic isoamylase were determined in 156 alcohol addicts during the first 9 days upon initiation of alcohol abstinence During this period decreasing activities were recorded for S-bilirubin and S-ASAT but patients possessing moderately increased serum activity of GT showed no decrease Increased activities of S-ASAT and S-GT were still frequently seen at the end of the observation period For S-pancreatic isoamylase both decreases and increases from initial activities were frequently found In patients with low activities initially, the S-pancreatic isoamylase activity increased during the observation period and thus, pathologically increased activities were recorded more often 9 days after alcohol withdrawal than on arrival at the hospital

Key words alcoholism aspartate aminotransferase bilirubin gamma-glutamyltransferase isoamylase

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Increased serum activities of aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) as well as γ glutamyltransferase (GT) are frequently seen in alcohol addicts (1 3 7 8 15 18 22) The elevation of S-ASAT is regularly more pronounced than that of S-ALAT (8 16 18) It is also known that the serum activities of ASAT and ALAT may increase for some days after ethanol intake Specific determination of the pancreatic isoamylase activity in serum has disclosed decreased levels to be a common finding in chronic alcoholics on admission to hospital (18)

The present study was undertaken to disclose successive changes in the serum levels of bilirubin ASAT GT and pancreatic isoamylase in a series of alcoholics upon initiation of alcohol abstinence

PATIENTS AND METHODS

The patient series consisted of 156 male inpatients at the Department of Alcohol Diseases Malmo General Hospital aged 25–70 years (mean 44) They had been hospitalized after a period of heavy alcohol consumption During hospitalization the patients were treated with a high protein–calorie diet vitamin injections (Parentrovite®) as well as nitrazepam (Mogadon®) clomethiazole (Heminevrin®) promethazine (Lergigan®) and dixyrazine (Esucos®) in selected cases None of the patients developed delirium tremens or had clinical or biochemical signs of liver cirrhosis

Blood samples were drawn immediately after arrival at the hospital before any treatment was instituted as well as every third day during the hospitalization The laboratory parameters studied were S-bilirubin S-ASAT S-GT S-salivary and S-pancreatic isoamylase and S-albumin Albumin was determined in a Technicon AutoAnalyzer with bromcresol green the other techniques used were as described previously (18) The patients were grouped into five or six classes on the basis of their initial laboratory values and each class was followed separately For S-bilirubin S-ASAT and S-GT the classes were normal range 1–2 3–5 5–10 10–20 and more than 20 times the values of the upper normal limit for S-pancreatic isoamylase increased activity normal range except its lowest limit lowest limit of normal range moderately and severely decreased activity (Fig. 1)

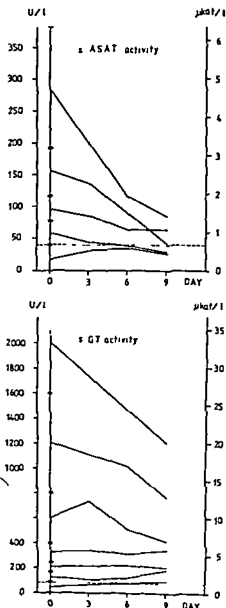
RESULTS

On arrival at the hospital 15% of the patients had an elevated serum concentration of bilirubin i e above 20 μmol/l (1 2 mg/100 ml) At the end of the observation period of 9 days S-bilirubin was elevated in 1 3% of the patients

The serum activity of ASAT was increased initially in 70% of the patients During the observation period the mean activities of the various groups decreased (Fig 1) However only patients with moderate initial increase showed a complete normalization Thus 36% of the patients still had a

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Abbreviations ASAT=aspartate aminotransferase ALAT=alanine aminotransferase GT=γ-glutamyltransferase



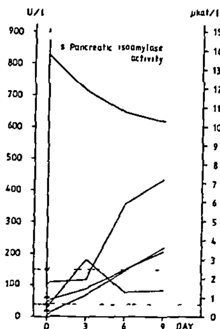


Fig 1 Changes upon initiation of alcohol abstinence in the mean serum concentrations of ASAT GT and pancreatic isoamylase Class ranges are indicated

pathologically high S-ASAT activity after 9 days of ethanol abstinence

Initially 66% of the patients had an increased activity of S-GT During the observation period there was no change in the groups whose initial activity had been slightly increased Decreasing activities however were recorded in patients with initially high S-GT activities i e more than 5 times the upper normal limit (Fig 1) After 9 days of abstinence 74% of all patients had an increased S-GT activity

The serum activity of pancreatic isoamylase was initially increased in 15% and decreased in 15% of the patients During the abstinence period there was a decrease in the patients with initially elevated values (Fig 1) However patients whose initial pancreatic serum isoamylase activity was low or normal showed a rise during the abstinence period at the end of which 34% had a pathologically increased activity and only 5% a decreased activity No correlation was found between pancreatic isoamylase activity and activities of S-ASAT or S-GT

The salivary isoamylase activity in serum which did not show any significant changes during the observation period was unrelated to the initial pancreatic isoamylase activity

Nor did the serum level of albumin show any

significant changes during the observation period The mean serum albumin concentration on admission was not correlated to the initial pancreatic serum isoamylase activity The albumin levels of the various groups (of S pancreatic isoamylase activity) were 43 43 45 43 and 44 g/l respectively

DISCUSSION

The initial frequencies of pathological serum levels of the studied parameters are in good agreement with earlier reports (7 8 11 12 14 15 18 21 22 23) The results of the present study clearly show that although there was a tendency towards normalization of increased activities for S ASAT and S-GT during the observation period i e the first 9 days of abstinence only patients with moderately increased serum activities normalized their serum levels Patients with higher initial values remained on an elevated level even at the end of that period This was especially the case for S-GT Thus it can be concluded that determination of S-GT at least 9 days after alcohol withdrawal can still be used as a measure of previous heavy alcohol consumption An elevated S-ASAT activity 9 days after withdrawal of alcohol indicates that the initial activity presumably would have been more than twice the upper normal limit

Patients who initially displayed laboratory signs of acute pancreatic involvement i e increased pancreatic serum isoamylase activity showed a tendency towards normalization of their activity However the activity decreased more slowly than is commonly seen in cases of acute pancreatitis In the latter cases as well as in the acute hyperamylasemia following endoscopic retrograde pancreatography a complete normalization is usually seen in about 5 days (19) The delayed normalization seen in the present patients may be related to the finding of a successive increase in the pancreatic serum isoamylase activity in the patients who initially had low or normal levels This finding may be interpreted as a depressing effect of ethanol on the pancreatic secretion i v ethanol administration is known to reduce the pancreatic output of enzymes in humans (9) Another explanation may be the deficient nutrition during the drinking period which is known to occur in about one third of chronic alcoholics Thus a reduced pancreatic serum isoamylase activity can frequently be recorded in patients with severe malnutrition (17) and pancreatic dysfunction is well documented in protein–calorie malnutrition (2 13 20) Mezey and Potter (10) found a normalization of the pancreatic trypsin output in chronic alcoholics following adequate diet despite continued ethanol intake The absence of a positive correlation between the albumin concentration and pancreatic isoamylase activity in serum on admission however supports the former explanation Nor was there any positive correlation between the salivary and the pancreatic serum isoamylase activities

As the patients in the present study were grouped according to their initial enzyme activities it was possible to demonstrate the very slow reduction of slightly and moderately increased activities of S ASAT and S-GT No significant delayed increase in the serum levels of ASAT or GT could be demonstrated The delayed increase in the serum activity of pancreatic amylase could be demonstrated because specific determination of the pancreatic serum isoamylase activity was performed and the patients were divided into classes based on the initial levels of their serum activities

REFERENCES

1 Bang N U Iversen K Jagt T & Madsen S Serum glutamic oxaloacetic transaminase activity in acute and chronic alcoholism JAMA 168 156 1958
2 Barbezat G O & Hansen J D L The exocrine pancreas and protein-calorie malnutrition Pediatrics 42 77 1968
3 Brohult I Carlson L A & Reichard H Serum-enzyme activities cholesterin and triglycerides in serum after intake of alcohol Scand J Clin Lab Invest (Suppl) 92 82 1966
4 The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology Recommended methods for the determination of four enzymes in blood Scand J Clin Lab Invest 33 291 1974
5 Jellinek E M The withdrawal syndrome in alcoholism Can Med Assoc J 81 536 1959
6 — The disease concept of alcoholism Hillhouse Press New Haven 1960
7 Johansson B G & Medhus A Increase in plasma α lipoproteins in chronic alcoholics after acute abuse Acta Med Scand 195 273 1974
8 Konttinen A Hartel G & Louhija A Multiple serum enzyme analysis in chronic alcoholics Acta Med Scand 188 257 1970
9 Marin G Ward N & Fischer R Effect of ethanol on pancreatic and biliary secretions in humans Am J Dig Dis 18 825 1973
10 Mezey E & Potter J Changes in endocrine

pancreatic function produced by altered dietary protein intake in drinking alcoholics Johns Hopkins Med J 128 7 1976
11 Myrhed, M Alcohol consumption in relation to factors associated with ischemic heart disease Acta Med Scand (Suppl) 567 1974
12. Myrhed M & Bergstrom, K Några leverenzymer hos alkoholdiskordanta tvillingpar In. Annual meeting of the Swedish Society of Medical Sciences Stockholm 1973
13 Pelaez, M J Gonzales P A & Velez A H Estudio de la funcion pancreatica en pacientes desnutridos. Antioquia Med 16 41 1966
14 Rosalki S B Screening test for alcoholism. Lancet 2 843 1973
15 Rosalki S B & Rau D Serum γ-glutamyl transpeptidase activity in alcoholism. Clin Chim Acta 39 41 1972
16 Salum, I Delirium tremens and certain other acute sequels of alcohol abuse Acta Psychiatr Scand (Suppl) 235 1972
17 Skude G Unpublished results
18 Skude G & Wadstein J Amylase hepatic enzymes and bilirubin in serum in chronic alcoholics Acta Med Scand 201 53 1977
19 Skude G Wehlin L Maruyama, T & Ariyama, J Hyperamylasemia after duodenoscopy and retrograde cholangiopancreatography Gut 17 127 1976
20 Tandon B N George P K Sama S K Ramachandran K & Gandhi P C Exocrine pancreatic function in protein calorie malnutrition disease of adults. Am J Clin Nutr 22 1476 1969
21 Thaler H Alkohol und Leberschaden Dtsch Med Wochenschr 23 1213 1969
22 Wallerstedt S & Olsson R. Personal communication
23 Wallgren H & Barry III H Actions of alcohol parts I-II Elsevier Amsterdam London and New York 1970

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Serum Ethanol, Hepatic Enzymes and Length of Debauch in Chronic Alcoholics

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ABSTRACT The serum concentration of ethanol and the activities of aspartate aminotransferase alanine aminotransferase and γ glutamyltransferase (GT) in 40 male chronic alcoholics were determined on admission to hospital The serum activities of the enzymes were highest in patients with established alcoholism for less than 5 years The serum concentration of ethanol, however, was lowest among these patients and gradually increased with the duration of alcoholism No correlation was found between the serum ethanol level and the activity of any of the enzymes The duration of the current debauch, which was shortest in cases of long standing alcoholism showed a positive correlation with the S GT activity

Key words alcoholism alanine aminotransferase aspartate aminotransferase gamma glutamyltransferase

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Increased serum activities of aspartate aminotransferase (ASAT GOT) alanine aminotransferase (ALAT GPT) and γ glutamyltransferase (GT) in chronic alcoholics have been reported by several authors (1 3 8 11 12 14 15 16 18 21 23) The relation of the enzyme activities to the current debauch has however not yet been established The present paper evaluates the serum activities of these enzymes in relation to the serum ethanol concentration on arrival at hospital and the duration of the current debauch in chronic alcoholism of varying duration as well as the relation between serum ethanol concentration and duration of the disease

PATIENTS AND METHODS

The patient series consisted of 40 male inpatients aged 25–60 years at the Department of Alcohol Diseases All patients had been brought to hospital in an unconscious state due to acute ethanol intoxication and all fulfilled the criteria of γ alcoholism (5 6) They were divided into 3 groups according to the duration of alcoholism 0–5 5–10 and more than 10 (mean 18) years The mean number of hospitalizations at the clinic for the various groups as well as the mean number of attacks of delirium tremens during the last 5 years are given in Table I The mean duration of the current debauch given in Table I is based on the anamnesis

Blood samples were drawn on admission The serum ethanol concentration was determined by gas chromatography and the enzyme activities were determined according to the recommendations of the Scandinavian Enzyme Committee (4) Using a reference group of 97 subjectively healthy adult males without previous control of their drinking habits the upper normal limit (mean + 2 S D) was found to be 0 65 μkat/l for S ASAT 0 85 for S ALAT and 1 2 for S-GT (18)

RESULTS

All three groups of alcoholics had significantly increased serum activities of all three enzymes The activity of S-GT was highest (mean 4 5 μkat/l) in patients with a duration of alcoholism of 0–5 years and decreased gradually with increasing duration (Fig 1) The same general pattern was found for S ASAT while the changes were less pronounced and not significant for S ALAT (Fig 1)

The concentration of S ethanol on the other hand showed the lowest mean 61 mmol/l (2 8‰) in the group with 0–5 years duration of alcoholism After 5–10 years of alcoholism the mean concentration was 71 mmol/l (3 3‰) and in the group with more than 10 years history of alcoholism the mean value reached 80 mmol/l (3 6‰) The duration of the current debauch for the various groups was 8 10 and 4 weeks respectively (Table I)

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Abbreviations ASAT=aspartate aminotransferase ALAT=alanine aminotransferase GT=γ glutamyltransferase

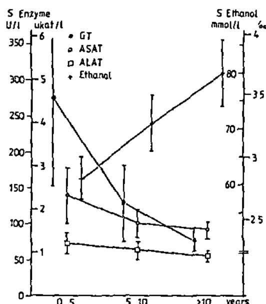


Fig 1 Serum activities of GT ASAT and ALAT and serum concentration of ethanol in relation to duration of chronic alcoholism (mean ± S E M)

Table I *Characterization of the patient groups*

	Years of chronic alcoholism		
	0–5	5–10	>10 (mean 18)
No of pats	10	12	18
Mean age (y)	39	42	49
Admissions for detoxication (*n*)	1 5	1 1	1 6
Attacks of delirium tremens (*n*)	0 8	0 8	2 1
Mean duration of current debauch ± S D (weeks)	8±2	10±2	4±2

Taking all three groups together the mean S GT activity was within the reference range when the debauch period was shorter than 2 weeks just above the reference range with a debauch of 2–6 weeks and markedly increased when the drinking period exceeded 6 weeks the activities being 0 65 55 and 5 80 μkat/l respectively No correlation was found between the S ASAT or S ALAT activities and the duration of the debauch Nor was there any correlation between the enzyme activities and the ethanol concentrations in serum Thus at a S-ethanol level of 65 mmol/l (3 0‰) the S GT activity varied from 0 3 to 9 0 μkat/l just as it did at an S-ethanol concentration of 137 mmol/l (6 4‰)

DISCUSSION

The high frequency of increased serum activities of the enzymes investigated is reflected by the high mean values The rise was most pronounced for S-GT followed by S ASAT and least for S ALAT which is in accordance with earlier reported values (8 16, 18) A tendency found earlier towards normalization of the serum activities after prolonged alcoholism (18) was confirmed in the present study This finding may have to do with the observation that the S GT levels were related to the duration of the current debauch The mean duration of the debauch was shortest in cases with long standing alcoholism (Table I) Thus the tendency towards normalization of the laboratory parameters in long standing alcoholism is probably an expression of a short debauch rather than an indicator of increased individual resistance to the toxic effects of ethanol as was proposed in the earlier report (18)

The finding of a short duration of the current debauch in combination with the highest S-ethanol concentrations in patients with more than 10 years history of alcoholism could indicate that subjects belonging to this group drink more heavily for a shorter period than those with a briefs history of alcoholism and/or that they have a less effective ethanol degradation and already need hospitalization after a rather short debauch

The main factor behind the rise of the S GT activity is not a high serum concentration of ethanol but a prolonged intake of alcohol

References are given in the preceding paper

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Maintenance of Potassium Balance during Diuretic Therapy

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ABSTRACT The relative efficacies of potassium chloride, amiloride, triamterene and spironolactone in maintaining potassium balance were studied in 40 patients with essential hypertension receiving diuretic therapy The preparations were administered in random order in a cross over manner In 31 patients treated with 50 mg hydrochlorothiazide daily, addition of 1500 mg potassium chloride daily was the weakest and 50 mg spironolactone daily the most effective agent for maintaining serum potassium Amiloride (5 mg daily) and triamterene (75 mg daily) were less effective and equally so Similar results were obtained with 9 patients treated with double dosages of the diuretic and supplements Despite changes in serum potassium, total body potassium remained constant throughout the trial

Key words hypertension serum potassium total body potassium diuretic therapy potassium supplementation potassium sparing agents

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Maintenance of potassium during diuretic therapy continues to be a topic surrounded by controversy There is disagreement on when supplementation should be started the most appropriate agents to be used and their dosages The purpose of this study was to evaluate the relative efficacies of potassium supplementation (PS) with potassium chloride (KCL) and certain potassium sparing agents (PSA) in maintaining serum and total body potassium (TBP) in a group of hypertensive subjects on diuretic therapy To minimize parameter variations a simple cross over technique was employed and the dose of diuretic held constant throughout all trial periods

PATIENTS AND METHODS

Forty patients with uncomplicated hypertension—25 men and 15 women mean age 52 years (range 41–70)—were studied Fifteen were overweight (10% or more above their ideal weight) All patients fulfilled the criteria of the WHO I group Before entering the trial all had been treated with antihypertensive agents for 1–6 years 17 with diuretics and PSA 15 with diuretics and PS 6 with diuretics alone and 2 with other antihypertensive agents only Eleven patients used a β blocker in addition (9 pindolol and 2 alprenolol) 2 took clonidine and 6 methyldopa

On admission to the trial serum potassium (SP) levels were 3 5 mmol/l or more (mean 4 5) There was an initial 3 month run up period for which all PS or PSA were stopped Hydrochlorothiazide (Hydrex®) 50 mg in a single daily dose was given to 31 patients while the other 9 were treated with 50 mg b i d These dosages were kept constant for all subsequent trial periods Other antihypertensive agents were held at uniform dosages throughout the trial At the end of the run up potassium parameters were determined and the patients entered a 3 week placebo period without diuretics In view of the possibility of increases in blood pressure a longer placebo period was considered unethical Patients were then assigned to a series of treatments consisting of either KCL supplementation or PSA in addition to their run up dosage of hydrochlorothiazide For the 31 patients treated with 50 mg hydrochlorothiazide the daily doses were amiloride 5 mg once daily spironolactone 25 mg b i d potassium chloride 750 mg b i d and triamterene 75 mg once daily Amiloride and triamterene were given in the form of combination tablets with hydrochlorothiazide (Diurex® and ONR 106 Ti respectively) whereas spironolactone (Spirodon®) and potassium chloride (Kaliduron®) were given separately in conjunction with the hydrochlorothiazide tablets Nine patients treated with 50 mg hydrochlorothiazide b i d also received double doses of KCL or PSA Each course of treatment lasted for 3 months except in cases in whom hypokalemia was detected After completion of each study period the patients spent 3 weeks on placebo tablets

All underwent a physical examination before commencement of the therapy and after each treatment period The purpose of the study was explained and only those willing to volunteer with good compliance were selected The patients consulted the same experienced

Abbreviations PS = potassium supplementation KCL = potassium chloride PSA = potassium sparing agents TBP = total body potassium SP = serum potassium PB = potassium balance

Table I. *Serum urate (mmol/l) in uncomplicated hypertonic patients at the end of various treatment periods*

	Hydrochlorothiazide	Hydrochlorothiazide + KCL	Hydrochlorothiazide + amiloride	Hydrochlorothiazide + triamterene	Hydrochlorothiazide + spironolactone	Placebo
Total						
Mean	0.359	0.358	0.356	0.347	0.328	0.266
S.E.	0.015	0.017	0.018	0.018	0.018	0.014
p	0.001	0.001	0.001	0.001	0.01	–
Men						
Mean	0.403	0.383	0.394	0.388	0.370	0.314
S.E.	0.017	0.018	0.023	0.020	0.023	0.014
*p**	0.001	0.001	0.001	0.001	0.01	–
Women						
Mean	0.298	0.270	0.295	0.282	0.266	0.239
S.E.	0.021	0.021	0.021	0.025	0.021	0.023
p	0.001	NS	0.001	0.05	NS	–
Single dose						
(mean ± S.E.)	0.349±0.017	0.329±0.019	0.336±0.019	0.329±0.018	0.317±0.020	0.281±0.016
Double dose						
(mean ± S.E.)	0.404±0.030	0.370±0.031	0.439±0.038	0.422±0.042	0.374±0.039	0.302±0.025
*p**	NS	NS	0.05	0.05	NS	NS

When compared to the placebo value *t*-test, dependent.
* *t*-test, independent.

...nurse throughout the trial. During the trial, patients were asked to maintain their normal life-styles.

SP, sodium, creatinine and urate, and TBP were determined at 3-month intervals. During the run-up period with hydrochlorothiazide alone, SP was determined every two weeks. Routine clinical methods were applied for ...ng serum electrolytes, creatinine and urate. TBP ...s measured by whole-body counter assessing ^{40}K with ...6 MeV γ-ray. Normal limits for SP concentration were taken to be 3.6–5.5 mmol/l.

RESULTS

All patients completed the trial and no subjective discomfort was reported. There were no statistically significant changes in blood pressure during the active treatment periods.

Serum sodium remained within normal limits throughout the trial. All patients had normal renal function as assessed by serum creatinine (below 115 µmol/l). Mean serum creatinine after the placebo period was 92.2±2.7 µmol/l. There were minor increases during the active trial periods, the highest rise (8.6 µmol/l) being observed during the triamterene combination stage. Mean serum urate concentration rose more markedly (Table I), the increase being clearer in males than females. In patients taking double doses of hydrochlorothiazide the increase in serum urate was slightly enhanced, notably during amiloride and triamterene combinations.

With the exception of the spironolactone combination, mean SP concentration was decreased during all active treatment periods (Table II, Fig. 1). The lowest value was obtained with hydrochlorothiazide alone. SP did not increase significantly from the hydrochlorothiazide value after administration of KCL, whereas following the treatment periods with PSA the mean SP level was significantly higher than during hydrochlorothiazide alone. However, spironolactone was the only agent able to raise mean SP to the same level as during the placebo period.

Hydrochlorothiazide alone rendered 8 patients (5 women, 3 men) hypokalemic, i.e. SP 3.5 mmol/l or less. Four of these patients were taking single doses and four double doses of the diuretic. Hypokalemia was detected 2–10 weeks after the beginning of the trial.

KCL supplementation was the least effective in elevating SP values. Three patients became hypokalemic while on this preparation. They also had low SP values while on hydrochlorothiazide alone.

During amiloride 2 female patients were rendered hypokalemic, with SP levels of 3.5 and 3.4 mmol/l. Both of them had similar low levels of SP while on

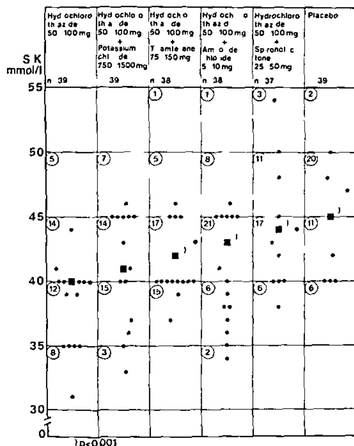


Fig 1 Individual SP values (●) during the different treatment stages ■ = Mean values Differences are calculated between the hydrochlorothiazide and the other periods

hydrochlorothiazide alone However during KCL both had normal SP

Spironolactone and triamterene prevented the development of hypokalemia while spironolactone raised SP to the upper limit of normal (5 3 mmol/l) in 3 patients

Double doses caused clearer decreases in SP except with spironolactone (Table III) The falls were statistically significant with oral potassium or triamterene administration

The patients whose SP concentrations had fallen to 3 5 mmol/l or less by the end of the hydro-

Table II *SP levels (mmol/l) in uncomplicated hypertonic patients at the end of various treatment periods and statistical significance of differences compared with hydrochlorothiazide alone*

	Hydrochloro thiazide (H)	Hydrochloro thiazide + KCL (H+K)	Hydrochloro thiazide + amilonde (H+A)	Hydrochloro-thiazide + triamterene (H+T)	Hydrochloro thaizide + spirono-lactone (H+S)	Placebo (P)
Mean	4 00	4 09	4 29	4 23	4 44	4 50
S E	0 07	0 07	0 06	0 06	0 07	0 06
H	–	NS	0 001	0 001	0 001	0 001
H+K	NS	–	0 01	NS	0 005	0 001
H+A	0 001	0 01	–	NS	NS	0 025
H+T	0 001	NS	NS	–	0 025	0 001
H+S	0 001	0 005	NS	0 025	–	NS
P	0 001	0 001	0 025	0 001	NS	–

t test dependent

Table III *SP (mmol/l) in uncomplicated hypertonic patients at the end of different treatment periods (mean ± S E)*

	Single dose	Double dose	p^a
Hydrochlorothiazide	4 04±0 07	3 86±0 17	NS
KCL + hydrochlorothiazide	4 18±0 08	3 82±0 12	<0 05
Amiloride + hydrochlorothiazide	4 32±0 07	4 16±0 19	NS
Triamterene + hydrochlorothiazide	4 30±0 06	3 98±0 08	<0 05
Spironolactone + hydrochlorothiazide	4 41±0 07	4 55±0 16	NS
Placebo	4 54±0 07	4 36±0 12	NS

[a] *t* test independent

chlorothiazide run in treatment had lower serum concentrations after the other periods as well except when spironolactone was administered (Table IV)

TBP remained constant throughout the trial (Table V) In patients rendered hypokalemic during the hydrochlorothiazide period there was a trend for TBP to be lower than in those remaining normokalemic although the difference was not significant (Table VI) Interestingly neither KCL nor PSA were able to increase TBP in these patients

DISCUSSION

The aim of the study was to compare the relative efficacies of KCL and the most commonly used A in maintaining body potassium balance (PB) ring diuretic therapy in subjects with stable, uncomplicated hypertension To minimize parameter variations a random cross over technique was employed using the subjects as their own controls during the different stages A long term trial would have been desirable but for practical reasons a cross over study with several successive 3 month treatment stages was selected This time was considered adequate for comparing the relative efficacies of various treatments in maintaining PB The altered PB induced by diuretic therapy appears to stabilize in 1–2 months (8) and to remain stable for years (7)

The number of tablets varied between the various stages of the trial KCL and spironolactone were given separately amiloride and triamterene in combination with hydrochlorothiazide One might attribute the poor serum response to KCL supplementation to poorer compliance in taking the tablets because of the greater number involved But this is hardly likely in view of the good efficacy of spironolactone which was taken in the same number of tablets

Our results indicate that in patients treated with daily doses of 50 mg hydrochlorothiazide KCL given in daily doses of 1 500 mg does not appear to be as effective as 5 mg amiloride 50 mg spironolactone or 75 mg triamterene Similar results were obtained with double doses of the drugs It has been suggested earlier that between 1 500 mg (5) and 6 g (4) of KCL daily is needed to safely maintain PB in patients with uncomplicated hypertension during diuretic therapy (2 4 5 8 9) Fluctuations in dosage duration of diuretic therapy the physical con

Table IV *SP (mmol/l) in patients rendered hypokalemic during the hydrochlorothiazide period and in other patients (mean ± S E)*

	Others	Hypokalemics	p^a
Hydrochlorothiazide	*4 16±0 05*	*3 39±0 05*	<0 001
KCL + hydrochlorothiazide	4 20±0 07	3 68±0 09	<0 01
Amiloride + hydrochlorothiazide	4 38±0 06	3 93±0 17	<0 01
Triamterene + hydrochlorothiazide	4 31±0 06	3 94±0 07	<0 01
Spironolactone + hydrochlorothiazide	4 48±0 08	4 27±0 06	NS
Placebo	4 53±0 06	4 33±0 18	NS

[a] *t* test independent

Table V *TBP (g) in uncomplicated hypertonic patients at the end of various treatment periods*

	Hydrochloro-thiazide	Hydrochloro-thiazide + KCL	Hydrochloro thiazide + amiloride	Hydrochloro-thiazide + triamterene	Hydrochloro thiazide + spironolactone	Placebo
Mean	123 2	123 0	124 8	122 4	124 1	125 2
S E	4 1	3 9	4 3	4 2	4 2	4 1

There were no statistically significant differences between various treatment periods (*t* test dependent)

dition and diet of the patients do not allow direct comparisons with previous investigations though the results of this study indicate that more than 1 500 mg KCL daily is needed to maintain PB

As expected serum urate concentrations were increased more in males than in females The in crease was enhanced with double doses of the drugs However no signs of clinical gout were observed

TBP remained unchanged as expected The use of KCL or PSA was started before signs of potas sium deficiency or depletion developed Further more most previous studies have shown that de spite falls in SP TBP remains constant during long term treatment with diuretics in uncomplicated hypertension (3 10) Controversial results have been reported where the treatment of hypertension with a diuretic led to reduction of TBP (5) or to falls in exchangeable potassium (1 6) Variations in the methods used for the determination of PB in the clinical state of the patients and in the diuretics used in different dosages do not allow direct compari sons Also some reports showing decreases in TBP during diuretic therapy have concerned patients with signs of cardiac decompensation (9) One could speculate that the 3 month periods in our trial were too short for fall in TBP to become detectable This is unlikely because TBP in this trial was not in creased during PS or after administration of PSA in patients rendered hypokalemic during the hydro-chlorothiazide stage which was followed by four active 3 month stages for improving PB

No hyperkalemic values occurred in the present study This means that administration of KCL and PSA to patients with uncomplicated hypertension in such doses as we used is possible and safe Only during spironolactone were values approaching the upper normal limit occasionally seen The Finnish diet tends to be poor in fruit and vegetables Whether this is related to the negligible incidence of hyperkalemia remains to be investigated

In the few patients developing hypokalemia while on hydrochlorothiazide alone SP was also low either during KCL or amiloride or both It was not possible to detect any special clinical signs indicat ing a tendency to falls in SP during diuretic therapy This implies a continuing general need for monitor ing the requirement for PS or PSA In patients treated with a daily dose of 50 mg hydrochloro-thiazide administration of 50 mg spironolactone daily maintained SP at desirable levels though in practical terms the response to 5 mg amiloride or 75 mg triamterene was almost as good Individuals needing correction of PB while on a diuretic alone or also receiving KCL or PSA can only be found by trial and error

Table VI *TBP (g) in patients rendered hypokalemic during hydrochlorothiazide period and in other patients (mean ± S E)*

	Others	Hypokalemics
Hydrochlorothiazide	126 4±4 3	110 9±10 4
KCL + hydrochlorothiazide	*125 2±4 2*	*114 3± 9 2*
Amiloride + hydrochlorothiazide	127 9±4 5	113 3±11 3
Triamterene + hydrochlorothiazide	125 2±4 6	112 1± 9 6
Spironolactone + hydrochlorothiazide	125 6±4 3	115 4±15 4
Placebo	127 1±4 1	116 7±13 1

There were no statistically significant differences between various treatment periods (*t* test independent)

Table III. *SP (mmol/l) in uncomplicated hypertonic patients at the end of different treatment periods (mean ± S.E.)*

	Single dose	Double dose	*p*
Hydrochlorothiazide	4.04±0.07	3.86±0.17	NS
KCL + hydrochlorothiazide	4.18±0.08	3.82±0.12	<0.05
Amiloride + hydrochlorothiazide	4.32±0.07	4.16±0.19	NS
Triamterene + hydrochlorothiazide	4.30±0.06	3.98±0.08	<0.05
Spironolactone + hydrochlorothiazide	4.41±0.07	4.55±0.16	NS
Placebo	4.54±0.07	4.36±0.12	NS

* *t* test, independent.

chlorothiazide run-in treatment had lower serum concentrations after the other periods as well, except when spironolactone was administered (Table IV).

TBP remained constant throughout the trial (Table V). In patients rendered hypokalemic during the hydrochlorothiazide period there was a trend for TBP to be lower than in those remaining normokalemic, although the difference was not significant (Table VI). Interestingly, neither KCL nor PSA were able to increase TBP in these patients.

DISCUSSION

The aim of the study was to compare the relative efficacies of KCL and the most commonly used PSA in maintaining body potassium balance (PB) during diuretic therapy in subjects with stable, uncomplicated hypertension. To minimize parameter variations, a random cross-over technique was employed, using the subjects as their own controls during the different stages. A long-term trial would have been desirable, but for practical reasons a cross-over study with several successive 3-month treatment stages was selected. This time was considered adequate for comparing the relative efficacies of various treatments in maintaining PB. The altered PB induced by diuretic therapy appears to stabilize in 1–2 months (8) and to remain stable for years (7).

The number of tablets varied between the various stages of the trial. KCL and spironolactone were given separately, amiloride and triamterene in combination with hydrochlorothiazide. One might attribute the poor serum response to KCL supplementation to poorer compliance in taking the tablets because of the greater number involved. But this is hardly likely in view of the good efficacy of spironolactone, which was taken in the same number of tablets.

Our results indicate that in patients treated with daily doses of 50 mg hydrochlorothiazide, KCL given in daily doses of 1 500 mg does not appear to be as effective as 5 mg amiloride, 50 mg spironolactone or 75 mg triamterene. Similar results were obtained with double doses of the drugs. It has been suggested earlier that between 1 500 mg (5) and 6 g (4) of KCL daily is needed to safely maintain PB in patients with uncomplicated hypertension during diuretic therapy (2, 4, 5, 8, 9). Fluctuations in dosage, duration of diuretic therapy, the physical con-

Table IV. *SP (mmol/l) in patients rendered hypokalemic during the hydrochlorothiazide period and in other patients (mean ± S.E.)*

	Others	Hypokalemics	*p*
Hydrochlorothiazide	4.16±0.05	3.39±0.05	<0.001
KCL + hydrochlorothiazide	4.20±0.07	3.68±0.09	<0.01
Amiloride + hydrochlorothiazide	4.38±0.06	3.93±0.17	<0.01
Triamterene + hydrochlorothiazide	4.31±0.06	3.94±0.07	<0.01
Spironolactone + hydrochlorothiazide	4.48±0.08	4.27±0.06	NS
Placebo	4.53±0.06	4.33±0.18	NS

* *t* test, independent.

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Mycoplasma Pneumoniae Infection Associated with Affection of the Central Nervous System

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ABSTRACT A total of 371 patients with acute febrile, non bacterial affection of the CNS hospitalized between Nov 1, 1971, and June 1, 1976 were examined for Mycoplasma (M) pneumoniae infection Nineteen of the patients showed evidence of a current M pneumoniae infection 32 of a previous infection, and 320 no evidence In patients with a current infection due to M pneumoniae, suggestive evidence is presented that this agent might be involved in the pathogenesis of the neurological syndromes Compared to cases without the infection these cases and to a lesser degree those with a previous M pneumoniae infection showed an increased frequency of pathological values found by various laboratory and instrumental parameters and a slightly higher frequency of neurological sequelae Respiratory illness was present in only 11 of the 19 patients infected with M pneumoniae a classical respiratory tract pathogen The overall incidence of current M pneumoniae infections among patients with neurological syndromes was 5%, with a maximum of 10% during the 1972 epidemic This is a much higher figure than expected from a mere coincidence of the two conditions

Key words Mycoplasma penumoniae central nervous system

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The respiratory tract pathogen Mycoplasma (M) pneumoniae is one of the most common causes of primary non bacterial pneumonia in man The disease has a low mortality and complications are rare (7 8 12)

Over the past decade various symptoms from the central nervous system (CNS) associated with M pneumoniae infection have been reported including meningitis encephalitis cerebellitis myelitis polyradiculitis and acute psychosis (5 9 10 15 22 23 24) Although neurological involvement may be severe virtually complete recovery has been described as a characteristic feature of the disease However both serious sequelae and fatal cases have been reported (5 10 15 22 24)

In the present study patients with acute febrile non bacterial syndromes of the CNS were investigated with the aim of evaluating the association with M pneumoniae infection

PATIENTS AND METHODS

Patient selection and grouping

All patients admitted to the Department of Infectious Diseases Rigshospitalet Copenhagen with fever and acute symptoms from the CNS were studied except those with the diagnosis of bacterial meningitis The first part of the study was conducted in the period from Nov 1 1971 to June 1 1973 which encompassed an epidemic of M pneumoniae infection in this country This series A comprised 102 patients

In order to get a better estimate of the incidence of M pneumoniae infection among the CNS patients the study was extended from June 1 1973 to June 1 1976 a period which included the 1975 epidemic A total of 269 patients were selected by the same criteria in series B but only those with a positive M pneumoniae antibody test were studied in more detail In series B patients with a negative M pneumoniae antibody test were registered only with respect to age sex time and clinical diagnosis of the CNS affection

A *retrospective series* of 73 patients submitted to the Department of Infectious Diseases with acute neurological infections during an 11 month period before Nov 1971 was selected on the basis that blood specimens had been sent to the Enterovirus Department at Statens Serum institut The sera which had been stored frozen were tested for antibodies to M pneumoniae

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Abbreviations CNS=central nervous system CF=complement fixation IF=indirect immunofluorescence CA=cold agglutinin RSV=respiratory syncytial virus CMV=cytomegalovirus EBV=Epstein Barr virus

All patients in series A and B were grouped according to serological reactions in two tests for antibodies to M. pneumoniae, i.e. a complement fixation (CF) test and an indirect immunofluorescence (IF) test. In *group I* the diagnosis of current or recent infection with M. pneumoniae was based on either a significant rise (≥4-fold) in antibody titre or a titre of ≥1000 in one of the tests. Patients with a positive CF or IF test but not fulfilling the criteria of group I were referred to *group II* and were considered to have had a M. pneumoniae infection within the past year. All patients with negative CF and IF tests were placed in *group III* and were considered to be without any experience of the infection in recent years.

Virus diagnosis

A diagnosis of viral infection was based on either 1) the clinical picture, 2) a rise in titre of antibodies to virus antigens (a positive Paul Bunnell test in cases of infectious mononucleosis), 3) the isolation of virus from the faeces or the spinal fluid, or by combinations of such criteria.

Clinical diagnoses

The neurological syndromes were diagnosed by the following symptoms and signs.

Non bacterial acute meningitis. Acute fever, meningeal symptoms, 5–1000 cells per µl in the spinal fluid and no bacteria by microscopy or culture. Spontaneous recovery without antibiotic treatment.

Encephalitis acuta. Acute fever, signs of focal derangement of the function of the cerebrum, brain stem or cerebellum, usually connected with non bacterial meningitis.

Polyradiculitis acuta. Symmetrical diffuse second neuron pareses with slight or no spinal fluid pleocytosis except in combined syndromes, high spinal fluid protein.

Myelitis acuta. Sensorimotor paralysis below a given spinal level.

Neuropathia peripherica acuta. Acute peripheral neuropathy of other clinical character than the above ioned syndromes.

numbers refer to their consecutive primary regis- on admission. Patients not fulfilling the criteria excluded.

Serology

All sera were titrated for antibodies to M. pneumoniae by a CF test (13) and an IF test (12). In the CF test a titre of ≥64 and in the IF test a titre of ≥160 was considered positive.

A cold agglutinin (CA) test was performed on all sera as previously described (13). Titres of ≥64 were designated as positive.

Antibodies to the following agents were measured by conventional CF technique (figures in brackets indicate the number out of the 133 patients (the total of 371 patients minus group III of series B) in whom two or more sera could be tested): influenza virus types A, B and C [117], adenovirus [117], respiratory syncytial virus (RSV) [116], morbilli virus [117], herpesvirus hominis [118], parotitis virus [75] and Chlamydia trachomatis group antigen [115] as a test for ornithosis. Antibodies to cytomegalovirus (CMV) [50] were tested by a CF test, and specimens showing a titre of ≥8 were tested also by a neutralization test. Epstein Barr virus (EBV) antibodies [51] were assayed by IF employing acetone fixed EBV infected cells as antigen. Heterophil antibodies indicating infectious mononucleosis were determined by Paul Bunnell's test including Davidsohn's absorption [116]. The following colleagues have been responsible for serological tests and virus isolation attempts: C. H. Mordhorst, Ornithosis Department (antibodies to influenza virus, adenovirus, herpesvirus hominis, morbilli virus, RSV and Chlamydia), J. C. Siim, Department of Toxoplasmosis (parotitis virus), E. Kjems, Streptococcus Department (Paul Bunnel test), A. Godtfredsen and M. Pedersen, Enterovirus Department (virus isolations), all from Statens Seruminstitut, Copenhagen, J. Hesse, Department of Tumor Virology, Institute of Medical Microbiology, University of Copenhagen (EBV), H. K. Andersen, Institute of Medical Microbiology, University of Århus (CMV).

Antibodies to human brain tissue were measured in all patients of series A by a CF technique similar to that employed for mycoplasmal antibodies, using as antigen a suspension in barbital buffer of homogenized biopsy material from the temporal lobe of patients surgically treated for epilepsy.

The concentration of IgM in serum was measured by radial immunodiffusion technique (14) using a rabbit antihuman µ chain preparation (Dakopatts, Copenhagen) and an IgM reference standard (Tri Partigen®, Behringwerke, Germany). By testing 13 pools of sera from normal individuals of age groups 0–20 years (306 sera), blood donors (131 sera) and recruits (60 sera), a mean value of 1.007 mg/l was found.

Isolation of mycoplasmas

Isolation of mycoplasmas was attempted from the patient's throat by bedside inoculation of the swab to conventional media for classical mycoplasmas (13). After transport within a few hours to the Mycoplasma Laboratory the specimens were incubated, subcultured and observed for at least one month. Shepard's media (21) were used for ureaplasmas. Classical mycoplasmas were finally identified by tests for growth or metabolism inhibition by species specific rabbit antisera (19), while ureaplasmas were identified by their urease activity and colony morphology (21). Spinal fluids were taken to the Mycoplasma Laboratory and then cultivated for mycoplasmas in a similar way.

Isolation of virus

Isolation of virus from the faeces and/or spinal fluids was attempted in 122 of the 133 patients (total series minus group III B). Two patients in group I and seven in group II were not tested. Isolation procedures were conventional, employing newborn mice and primary monkey kidney cultures supplemented with HeLa cultures or primary human amnion cultures.

Statistical methods

For comparison of two proportions Fisher's exact test was used. Trends in proportions (group I to group II to group III) were evaluated by a normal distribution approximation to Wilcoxon's two-sample test with correction for ties. All tests quoted as $p =$ and $p <$ are one sided tests. Two-sided tests are denoted by $2p =$ or $2p <$.

Table I *Distribution of patients according to principal symptoms from the CNS and to groups I II and III of series A and B*

Group I M pneumoniae antibody titre ≥1 000 or rise in titre group II M pneumoniae antibody titre positive excluding group I group III M pneumoniae antibody titre negative

	No of pats						
		Series A			Series B		
Principal symptoms from CNS	Total	I	II	III	I	II	III
Meningitis lymphocytaria	253	6	4	54	4	15	170
Encephalitis acuta	68	3	3	11	3	5	43
Polyradiculitis acuta	42	0	2	16	2	0	22
Myelitis acuta	7	1	1	1	0	1	3
Neuropathia peripherica acuta	1	0	0	0	0	1	0
Total	371	10	10	82	9	22	238

RESULTS

M pneumoniae infections and CNS affections

The number of patients in series A and B and in groups I II and III respectively are given in Table I which also shows the distribution with respect to symptoms from the CNS There was no preponderance of the different syndromes for any particular group

The criteria of a current or recent M pneumoniae infection (group I) were fulfilled by 10 patients (9 8%) in series A and by 9 (3 3%) only in series B These 19 patients are listed in Table II which also shows the titres of M pneumoniae antibody together with symptoms from the CNS and the respiratory tract as well as CSF findings The principal diagnoses were lymphocytic meningitis in ten cases encephalitis in seven and polyradiculitis and transverse myelitis in one case each

In 10 of the 19 patients with a current M pneumoniae infection (group I) a rise in titre of specific antibodies was demonstrated on days 6–20 (average on day 12) after onset of CNS symptoms (Table II) In seven of the other nine group I patients with titres of ≥1 000 a significant fall in titre was demonstrated in the course of illness The remaining two (cases 6 and 347) were hospitalized for 9 and 8 days only which precluded the observation of a change in titre

Respiratory tract infection was present in 11 of the 19 group I patients including eight with pneumonia (comfirmed by X ray) (Table II) In these 11 patients CNS symptoms appeared on days 1–18 after onset of illness (average on day 6) In only three patients did the illness begin with respiratory symptoms while the first symptom was neurological in one general malaise in six and a rash in one Of the remaining eight patients who had neither recent nor current respiratory infections the first symptoms were neurological in six while general malaise started in two 2 and 14 days respectively (average 2 8 days) before CNS symptoms appeared

Clinical syndromes and laboratory findings

There were no clinical syndromes specific for or characteristic of the CNS affections associated with M pneumoniae infection For some parameters however shown in Table III high values were measured relatively more often among group I than among group III patients with group II in an intermediate position With the degree of significance added in parentheses these parameters were ESR ($2p<0\ 05$) first count of peripheral blood leucocytes ($2p<0\ 01$) maximal temperature in the first 14 days ($2p<0\ 05$) and duration of fever ($2p<0\ 001$) Medium plus heavy changes in the electroencephalogram were likewise relatively more frequent among group I patients ($p<0\ 01$) Erythrocyturia or haemoglobinuria was more frequent in groups I and II than in group III ($p=0\ 018$) For other parameters such as spinal fluid findings no differences were detected between the three groups nor did symptoms from various organs like liver pancreas myocardium kidney or pericardium show predeliction for any particular group

Table II *Data on 19 patients in group I with M pneumoniae infection and symptoms from the CNS*

Series	Case no	Sex	Age (y)	Maximum M pneumoniae antibody titre CF	IF	Rise in titre on the day after debut of CNS symptoms	Principal CNS symptoms appearing on the day after onset of illness		Respiratory tract infection
A	1	♂	3	2 000↓	160		Meningoencephalitis ac	8	Pneumonia
A	2	♀	8	2 000↓	1 250		Myelitis transversalis	4	URI
A	3	♂	20	64↑	80	20	Encephalitis acuta	4	Pneumonia
A	6	♀	35	8 000	2 560		Meningitis lymphocytaria	18	Pneumonia
A	48	♀	18	128↑	40	7	Meningitis lymphocytaria	1	None
A	53	♂	5	64↑	<20	6	Meningitis lymphocytaria	5	Pneumonia
A	57	♀	6	1 000	320↓		Meningitis lymphocytaria	4	Pneumonia
A	62	♂	45	10 000↓	10 000↓		Meningitis lymphocytaria	16	Pneumonia
A	72	♂	7	256	320↑	9	Meningitis lymphocytaria	5	Pneumonia
A	69	♂	4	32↑	60↑	11	Encephalitis acuta	1	URI
B	155	♂	47	128↑	160	18	Meningoencephalitis ac	1	None
B	211	♂	62	512↑	80↑	14	Meningoencephalitis ac	2	None
B	287	♀	30	1 000↓	640↓		Encephalitis acuta	1	None
B	292	♀	19	2 000↑	2 560↑	9	Meningitis lymphocytaria	3	Pneumonia
B	329	♂	9	2 000↓	640↓		Meningitis lymphocytaria	1	None
B	333	♀	22	64↑	40	15	Polyradiculitis acuta	1	None
B	347	♀	8	1 000	640		Meningitis lymphocytaria	14	None
B	360	♂	9	64	160↑	17	Meningoencephalitis ac	1	None
B	440	♂	21	2 000↓	640		Meningitis lymphocytaria	6	URI

↑=Rise ↓=fall in titre URI–upper respiratory tract infection

For the sake of simplicity the distribution of patients as shown in Table I was made according to their principal symptoms from the CNS In some patients however symptoms could be referred to other sites of the CNS also Most cases of meningoencephalitis were registered as encephalitides In group I there were four such patients (Table II) one of whom had also a polyradiculitis (case 1) One in group II (case 39) had a radiculitis in tion to the main diagnosis of myelitis Among - patients in group III with encephalitis as the principal diagnosis one also suffered from myelitis another had cerebellitis while the third had symptoms from both the meninges and the radices

Sequelae

The severity of the CNS disease was estimated by the occurrence of neurological sequelae (Table IV) In series A there were four (20%) out of 20 patients with a present or past M pneumoniae infection (groups I + II) who developed sequelae which was the case in four (4 9% only) of the 82 patients without this infection (group III) a difference which is slightly significant ($p=0\ 046$) Altogether five out of 19 group I and four out of 32 group II patients had sequelae Group III patients in series B were not registered in this respect The syndromes leading to sequelae were encephalitis radiculitis and myelitis

Incidence

The distribution by trimesters of all 371 cases within the three groups is shown in Fig 1 upper graph which includes the 73 cases from an 11 month period prior to Nov 1971 Cases of M pneumoniae infection (group I) in series A accumulated in the winters of 1971–72 (four cases) and 1972–73 (six cases) The upper graph of Fig 1 shows a coincidence of these two peaks with the peaks of the lower graph registering the 1972 epidemic in this country (13) In series B from June 1973 to June 1976 group I cases did not accumulate corresponding to peaks of the epidemic occurring through 1975

Isolation of mycoplasmas

Attempts to isolate mycoplasmas from the throat and/or the CSF were carried out in 35 cases of series A Throat swabs from 24 of the patients including five in group I yielded mycoplasmas in 14 cases M pneumoniae was isolated only once namely from a group I patient (case 6) suffering from lymphocytic meningitis and pneumonia M salivarium was cultivated from the same swab

pinal fluid findings	
eucocytes (max no /µl)	Lymphocytes (%)
337	60
760	15
23	75
208	63
165	100
80	30
43	80
115	90
135	100
3	100
383	98
739	95
18	100
8	100
14	100
4	100
22	50
48	100
190	26

In the remaining 13 cases M salivarium was isolated from 10 M hominis and M orale type 1 from one patient each Ureaplasma urealyticum was isolated from the throat of one group I patient a 20-year-old man who showed a rise in titre of antibodies to both M pneumoniae and herpesvirus hominis (vide infra) Isolation of these mycoplasma species was not correlated to any particular group or syndrome

Spinal fluids from 19 patients including one from group I were cultivated for mycoplasmas all with negative results

Virus infections

A diagnosis of current virus infection was made in 46 of 133 patients (371 patients minus series B group III) Four of these patients belonged to group I 14 to group II and 28 to group III The four group I patients with viral infections were 1) A 20-year old man with encephalitis (case 3) who had a rise in titre of antibodies to herpesvirus hominis 2) A five year-old boy with lymphocytic meningitis (case 53) in whom echo virus type 7 was isolated from the faeces 3) A 22 year-old woman (case 333) in whom antibodies to morbilli virus developed during a polyradiculitis 4) A 62 year-old man (case 211) suf

Table III *Number of patients in each group in whom values indicated for each parameter were found*

Figures in parentheses denote number of patients from whom data were obtained

	Group		
	I (n=19)	II (n=32)	III (n=82)
ESR			
>50 mm/h	8 (18)	5 (32)	12 (81)
Peripheral blood leucocytes >8 500/µl in first count	14 (19)	15 (32)	29 (80)
Maximal rectal temperature first fortnight			
39 1–40 C	12 (19)	12 (31)	30 (81)
>40 C	5	7	8
Duration of temperature of >37 5 C			
One week	5	17	52
Two weeks	7 (19)	7 (28)	11 (70)
>two weeks	7	4	7
Changes in electro-encephalogram			
Light	1	4	15
Medium	5 (10)	4 (17)	13 (58)
Heavy	3	4	6
Erythrocyturia or haemoglobinuria	2 (19)	5 (32)	2 (80)

Table IV *Number of patients in each group with neurological sequelae after affection of the CNS*

	Series A			Series B	
Principal symptoms from CNS	I (n=10)	II (n=10)	III (n=82)	I (n=9)	II (n=22)
Polyradiculitis		1	2		
Myelitis ± encephalitis or radiculitis	1	1	1		
Encephalitis ± meningitis	1		1	3	2
Total	2	2	4	3	2

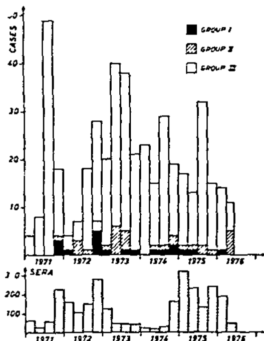


Fig. 1. Number of cases per trimester (upper graph) and number of M. pneumoniae antibody positive sera out of CA positive sera over the same period (lower graph) (8).

fering from a meningoencephalitis who had had rises in titre of antibodies both to Chlamydia and to herpesvirus antigens at the same time. All four patients showed a concomitant rise in titre of antibodies to M. pneumoniae.

Infection with parotitis virus, herpes zoster virus, adenovirus, Coxsackie and echo viruses was associated with lymphocytic meningitis only, and rubella and vaccinia viruses with encephalitis only. None of the other virus infections could be correlated to any specific neurological syndrome.

Serum IgM

The concentration of IgM in serum was measured in 126 of the 133 patients (all 371 minus series B, group III). Using the mean value of normal sera as a limit (see Patients and Methods) the number of patients whose sera showed values exceeding 1 000 mg/l was calculated. In group I 14 out of 17, in group II 26 out of 31, and in group III 39 out of 78 patients had a concentration of IgM above 1 000 mg/l. The figures for groups I and II are not significantly different; they all show IgM levels significantly higher than those of group III ($p=0.013$ and $p=0.0008$, respectively). The mean IgM concentration in group I was 2 937 mg/l (range 630–15 300), in group II 1 735 (range 630–5 030) and in group III 1 194 (range 310–5 940). Repeated IgM determinations were made mainly in groups I and II, where an increase or a decrease in IgM of more than 30% was found in nine group I cases and in five group II cases, including one case of infectious mononucleosis.

Autoantibodies

The CA test was positive in 38 out of the 133 patients and in about half of the patients with a positive M. pneumoniae antibody test (groups I and II). The CA titre in the 19 group I patients correlated neither with the titre of Mycoplasma antibodies nor with age or infection of the respiratory tract. In 13 of 82 group III patients, CA occurred both with and without a diagnosed virus infection.

Antibodies to human brain were looked for in all 102 patients of series A. In group I the patient with a transverse myelitis (case 2) had an anti brain CF titre of 256 on admission; 45 days later the titre had fallen to 16. Another group I patient (case 6) showed an anti brain titre of 1 000 together with an M. pneumoniae antibody titre of 8 000. This 35 year old woman was admitted for a lymphocytic meningitis and M. pneumoniae was isolated from her throat. In group II a 49 year-old woman with a polyradiculitis (case 13) had an anti brain titre of <16 ten days after onset of CNS symptoms and nine days later it was 16. In the remaining 99 patients all anti brain titres were <8.

Antibiotic treatment

Evaluation of the effect of antibiotic treatment was not planned. The appropriate antibiotics, tetracycline or erythromycin, were given to five patients in group I, including three with neurological sequelae, and to two patients in group II, one of whom developed sequelae. Tetracycline or erythromycin was not given to any of the 82 group III patients in series A.

DISCUSSION

This study raises two critical and integrated questions, namely whether the criteria used for the diagnosis of a current M. pneumoniae infection are sufficient and whether this infection is of any aetiological significance for the associated neurological symptoms.

A rise in titre of antibodies to M. pneumoniae in the second or third week of illness has not yet been

ascribed to other cross reacting infectious agents (7 12) A high titre generally indicates a current infection

Rising M pneumoniae antibody titres were demonstrated during the second to third week of neurological illness in 10 of the 19 group I patients This finding strongly indicates a pathogenetic connection of neurological symptoms to the M pneumoniae infection The high antibody titres found in the course of illness in the other nine group I patients and the significant fall in titre in seven of them also indicate a strong correlation between M pneumoniae infection and the CNS disease

Respiratory tract infection in 11 of the 19 group I patients including 8 with pneumonia was diagnosed both among those with rising titres (5 cases) and among those with high and falling titres (6 cases) (Table II) No particular syndrome was associated with respiratory symptoms

Patients with M pneumoniae infection show a significant increase in serum IgM concentration only part of which is represented by specific antibodies and CA (1 18 20) High IgM levels are also found in other acute infections among which infectious mononucleosis (25) and hepatitis (4) are the most common in Denmark The finding that the average serum IgM level in group I patients was significantly (2 9 times) higher than that of controls and about 2 5 times higher than that of group III patients contributes circumstantial evidence for the diagnosis of M pneumoniae infection The elevated IgM values of group II patients are in accordance with a past or current Mycoplasma infection

It is an unanswered question whether the associated neurological symptoms were generated by a Mycoplasma infection localized to the CNS or whether they were results of some maybe delayed immunological response of the CNS to Mycoplasma infection of the respiratory tract or of another site

Unfortunately attempts to isolate M pneumoniae from the throat or spinal fluid were not carried out on all patients Isolation of M pneumoniae from the spinal fluid has been reported in one case of polyneuritis (6) To our knowledge mycoplasmas have not been isolated from patients nervous tissues

The role of circulating anti brain antibodies in the pathogenesis of CNS symptoms is uncertain They were found at a significant level by CF in two of ten group I patients but not in the other 92 patients of series A However these antibodies which cross react with M pneumoniae have been found also in patients with Mycoplasma pneumonia without affection of the CNS (2) It is possible that another serological test showing reaction with different brain antigens might reveal a correlation with neurological symptoms in patients with M pneumoniae infection

M pneumoniae infection causes transient depression of T lymphocyte function measured as a decreased responsiveness in vitro of blood lymphocytes to PPD and to other unrelated antigens or by a transient negativity of the tuberculin skin test (3 16 17) Whatever the mechanism the result of this T cell depression may be that the mycoplasmas make their way to the CNS or alternatively clear the way for another infective agent causing CNS disease Double infections have been reported in M pneumoniae patients (7 11 22) In the present study a current virus infection was observed in three of the 19 group I patients and a fourth patient had indication of both herpesvirus and Chlamydia infection Among the 32 group II patients considered to have had a past M pneumoniae infection virus infection was diagnosed in 14 The total number of such cases may have been underestimated since in only a proportion of patients paired sera were available for virological serodiagnosis and faeces and spinal fluids were not examined for the presence of virus in all patients (see Patients and Methods) More data are needed to elucidate whether double infections are of any significance for the pathogenesis of neurological syndromes like those of the present study

The overall incidence of M pneumoniae infections among patients hospitalized for an acute febrile non bacterial affection of the CNS (group I) was 5 1% The incidence of M pneumoniae antibody positive patients (groups I and II) was 13 7% These figures are much higher than would be expected from a mere coincidence of the two conditions In a control material of 515 healthy individuals in age groups predisposed to symptomatic M pneumoniae infection there were from April 1971 to Aug 1975 15 (2 9%) with a CF antibody titre of 64 corresponding to group II and none corresponding to group I (13) In the present study an accumulation coincided with the 1972 epidemic covering 6 trimesters (Fig 1) (13) During this period the incidence of group I patients was 13 5% The 1975 epidemic was of comparable magnitude and extension but it was not accompanied by a similar ac

cumulated number of group I cases No explanation has been found for this discrepancy Possible factors as variations in virulence or neurotoxicity of the prevalent M pneumoniae strains or in the immune status of the population at various times have so far not been determined

It is concluded that patients in group I had an actual or recent (current) M pneumoniae infection Suggestive evidence for a causal association between this infection and the neurological syndromes is presented The pathogenesis of these syndromes however has not been clarified

REFERENCES

1 Biberfeld G Antibody responses in Mycoplasma pneumoniae infection in relation to serum immunoglobulins especially IgM Acta Pathol Microbiol Scand (B) 79 615 1971

2 — Antibodies to brain and other tissues in cases of Mycoplasma pneumoniae infection Clin Exp Immunol 8 319 1971

3 Biberfeld G & Sterner G Effect of Mycoplasma pneumoniae infection on cell mediated immunity Infection (Suppl) 4 17 1976

4 Dietz W H Jr Porcell O Moon T E Peters C J & Purcell H IgM levels and IgM mediated immune responses in patients with acute hepatitis A acute hepatitis B and chronic HB antigenemia Clin Exp Immunol 23 69 1976

5 Dorff B & Lind K Case report Two fatal cases of meningoencephalitis associated with Mycoplasma pneumoniae infection Scand J Infect Dis 8 49 1976

Fleischhauer P Huben U Mertens H Sethi K K & Thurmann D Nachweis von Mycoplasma pneumoniae im Liquor bei akuter Polyneuritis Dtsch Med Wochenschr 97 678 1972

7 Foy H M & Grayston J T Pneumonia Mycoplasma pneumoniae In Communicable and infectious diseases (ed F H Top & P F Wehrle) pp 480–494 Mosby St Louis 1972

8 Foy H M Kenny G E McMahan R Mansy A M & Grayston J T Mycoplasma pneumoniae pneumonia in an urban area JAMA 214 1666 1970

9 Klimek J J Russman B S & Quintiliani R Mycoplasma pneumoniae meningoencephalitis and transverse myelitis in association with low cerebrospinal fluid glucose Pediatrics 58 133 1976

10 Lerer R J & Kalavsky S M Central nervous system disease associated with Mycoplasma pneumoniae infection Report of five cases and review of the literature Pediatrics 52 658 1973

11 Lind K Isolation of Mycoplasma pneumoniae (Eaton agent) from patients with primary atypical pneumonia Acta Pathol Microbiol Scand 66 124 1966

12 — Mycoplasma pneumoniae infection Serological aetiological and epidemiological studies pp 1–130 F A D L s Forlag Copenhagen Århus and Odense 1973

13 Lind K & Bentzon M W Epidemics of Mycoplasma pneumoniae infection in Denmark from 1958 to 1974 Int J Epidemiol 5 267 1976

14 Mancini G Carbonara A O & Heremans J F Immunochemical quantitation of antigens by single radial immunodiffusion Immunochemistry 2 235 1965

15 Mårdh P A Ursing B & Lind K Case report Persistent cerebellar symptoms after infection with Mycoplasma pneumoniae Scand J Infect Dis 7 157 1975

16 Mizutani H & Mizutani H The skin reactive antigens of Mycoplasma pneumoniae Jpn J Microbiol 19 157 1975

17 Mogensen H H Andersen V & Lind K Lymphocyte transformation studies in Mycoplasma pneumoniae infection Infection (Suppl) 4 21 1976

18 Nordbring F Hogman C & Johansson S G O Serum immunoglobulin levels in the course of acute pneumonia Scand J Infect Dis 1 99 1969

19 Purcell R H Chanock R M & Taylor Robinson D Serology of the mycoplasmas of man In The mycoplasmatales and the L phase of bacteria (ed L Hayflick) pp 221–264 North Holland Publishing Company Amsterdam 1969

20 Schonell M E Immunoglobulin levels in pneumonia Clin Exp Immunol 8 63 1971

21 Shepard M C Cultivation and properties of T strains of mycoplasma associated with non gonococcal urethritis Ann NY Acad Sci 143 505 1967

22 Skoldenberg B On the role of viruses in acute infectious diseases of the central nervous system Clinical and laboratory studies on hospitalized patients Scand J Infect Dis (Suppl) 3 1 1972

23 Steele J C Gladstone R M Thanosophon S & Fleming P C Mycoplasma pneumoniae as a determinant of the Guillain Barré syndrome Lancet 2 710 1969

24 Sterner G & Biberfeld G Central nervous system complications of Mycoplasma pneumoniae infection Scand J Infect Dis 1 203 1969

25 Wollheim F A Immunoglobulin changes in the course of infectious mononucleosis Scand J Haematol 5 97 1968

Acta Med Scand) 333– 38 19 9

Immunoglobulin Deposits in the Dermo Epidermal Junction Zone

Nosograph c Occurrence ... her f Med cal D seases

Henrik Permin Frede Jurl nd Allan Wi k

From the Immunolog cal Labo a ory C ... Cl n c for Infect ous D eases R gshosp tlet C p n a n Denma k

ABSTRACT A material comprising 500 biopsies from clinically uninvolved skin of patients suffering from a number of medical diseases was studied by the direct immunofluorescence technique for immunoglobulins A M G D and E complements Clq and C3 as well as fibrinogen as deposits in the dermo-epidermal zone Granular deposits were found in about 3/4 of the biopsies from pat ents with systemic lupus erythematosus (SLE) rheumatoid arthritis patients suspected of having a connective tissue disease patients on medication known to induce at times an SLE syndrome and patients with diabetes mellitus As to infectious diseases and renal diseases deposits were found in about one half About 1/3 of patients with allergic diseases and malignancies had deposits Among patients with arteritis and among controls deposits were observed in about 10% In all groups but one diabetes mellitus the deposits were predominantly of the IgM class somewhat less commonly of IgG and rarely of the IgA or IgD classes Complement C3 and fibrinogen-antigenic material were also commonly found to have been deposited in connective tissue diseases drug-induced allergic and malignant diseases whereas Clq occurred mainly in SLE and never in endocrine diseases allergic diseases or in pat ents on medication IgG deposits were most often found in diabetes mellitus SLE and drug induced diseases The granular appearance and the content of the deposits indicate that they may represent immune complex deposition There was no correlation between immune deposits and the presence of an t nuclear antibod es or rheumatoid factors in the serum at the time of biopsy Thus skin biopsy from clinically normal skin studied for Ig and complement depos ts of the granular type does not essentially contribute to the differential diagnosis between a number of diseases within internal med cine The explanation is perhaps the presence of circulating immune complexes in several of these conditions

Ke wo ds dermo-ep dermal junct on zone immunoglobul n ant nuclear antibod es

Ac a M d Scand 05 333 1979

In 1963 Burnham et al (8) us ng a d rect fluorescence techn que reported a well demarcated fluorescent band of mmunoglobulins in the dermo-ep dermal (DE) junct on zone of sk n les ons from patients with systemic lupus erythematosus (SLE) In the following years this phenomenon was also observed in uninvolved skin from patients with SLE and certain other connective tissue diseases e g scleroderma and rheumatoid arthritis (RA) (4 5 7 10 1? 14 15 ?6) Sim lar depos ts of immunoglobul n have now been demonstrated also in cyst c f bros s lepromatous leprosy (?0 ??) and mult ple sclerosis (19)

The object of the present study was to establish the diagnostic value of studying skin biopsies for deposits of immunoglobul n and complement in a number of nfect ous and med cal diseases

PATIENTS AND METHODS

Table I lists the diagnoses in the 500 patients from whom skin b ops es were taken In 72 cases at least two b ops es were taken but only the result of the most clearly positive one s ncluded At the same time a serum sample was drawn from all pat ents These samples der ed p edominantly from departments taking a special interest n rheumatic and infectious d seases The diagnoses which will be discussed n more detail n reporting the results

Abb e a ons DE = dermo-ep dermal SLE system c lupus erythematosus RA rheumato d arthrit s PBS = phosphate buffered saline ANA = ant nuclear ant body ON organ non-spec f c GS granulocyte pec f c FITC fluorescein sothiocyanate

Table I *Prevalence of deposits in the dermo epidermal junction zone within the diagnostic groups*
Percentage of positivity is given in parentheses

Diagnosis	Positive/ total	IgA	IgM	IgG	IgD	Clq	C3	Fibrinogen
SLE	32/39 (82)	3 (8)	24 (62)	11 (28)	1 (3)	9 (23)	14 (36)	12 (31)
RA	29/59 (49)	0 (0)	21 (36)	1 (2)	0 (0)	7 (12)	7 (12)	4 (7)
Other connective tissue diseases	31/59 (53)	6 (10)	16 (27)	9 (15)	0 (0)	5 (8)	20 (34)	9 (15)
Arteritides	1/17 (6)	0 (0)	1 (6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Drug treatment	42/58 (72)	6 (10)	24 (41)	22 (38)	0 (0)	1 (2)	25 (43)	17 (29)
Infections	62/108 (57)	7 (6)	39 (36)	16 (15)	0 (0)	12 (11)	15 (14)	16 (15)
Endocrine diseases	22/32 (69)	2 (6)	6 (19)	16 (50)	0 (0)	0 (0)	10 (31)	11 (34)
Allergic diseases	10/27 (37)	1 (4)	7 (26)	3 (11)	0 (0)	0 (0)	7 (26)	9 (33)
Renal diseases	9/20 (45)	0 (0)	5 (25)	5 (25)	0 (0)	1 (5)	3 (15)	3 (15)
Malignancies	4/11 (36)	0 (0)	2 (18)	0 (0)	0 (0)	1 (9)	3 (27)	2 (18)
Ulcerative colitis	2/3	0	0	0	0	0	2	0
Miscellaneous diseases	5/56 (9)	0 (0)	5 (9)	1 (2)	0 (0)	0 (0)	3 (5)	2 (4)

were based upon the history clinical and laboratory results recorded in discharge cards and/or case records The biopsies were taken for research purposes and the results were not reported until later to the departments so that they have hardly influenced the diagnoses made or the wording of the discharge cards

Skin biopsies 4 or 5 mm in diameter were taken from the flexor aspect of the upper arm from normal looking skin by the punch technique after analgesia by ethyl chloride spray The specimens were immediately transferred to and stored at −70 C Within one week the specimen was cut in the cryostat into sections 4–6 μ thick The sections were air dried for 15 min washed in phosphate buffered isotonic saline (PBS) pH 7 2 for 30 min and incubated with 1 drop of diluted conjugate in a moist chamber for 30 min Thereafter they were washed twice in PBS and mounted with glycerol PBS and cover slips The preparations were read within 24 hours Sections without conjugate were used as controls for autofluorescence The specificity of positive findings was checked by blocking with corresponding unconjugated antiserum The results were considered positive if there was immunoglobulin and/or complement with or without fibrinogen antigenic material whereas the mere finding of the latter was not considered positive

Antibasement membrane antibodies were studied by the indirect immunofluorescence technique using normal human skin and guinea pig lip as antigenic materials Sera were studied in the dilution 1/16 and as conjugate we used polyspecific antihuman immunoglobulin Serum from a patient with bullous pemphigoid was included as a positive control while normal serum and PBS served as negative controls

Granulocyte specific and organ non specific antinuclear antibodies (GS and ON ANA) of the IgG class and their possible complement C3 fixing properties were studied by the indirect immunofluorescence technique (18 29 30 31)

Rheumatoid factors were studied by the RA latex agglutination technique (Behringwerke West Germany) Titres of ≥32 were considered abnormal

Conjugates

Fluorescein isothiocyanate (FITC)-labelled rabbit IgG specific for human α γ and μ chains and complement C3 as well as fibrinogen and a polyspecific antihuman immunoglobulin conjugate were obtained from Dakopatts Denmark Rabbit antisera specific for human δ and ε chains and complement Clq were obtained from Behringwerke West Germany The isolated IgG fraction of these antisera was labelled with FITC and the specificity was confirmed by immunoelectrophoresis and by the direct immunofluorescence technique (30 31) Results were read in a Leitz Orthoplan microscope equipped for incident light illumination as already described in detail (29)

RESULTS

Deposits of immunoglobulins fibrinogen and complement in the DE zone found in the skin biopsies are listed in Table I Deposits of IgE were not found in any patient

Systemic lupus erythematosus The SLE group comprised 39 patients who fulfilled at least 4 of the 14 criteria set up by the American Rheumatism Association (9) 28 females and 11 males with a mean age of 48 years (range 13–81) Deposits in the DE zone were found in 32 of them The most commonly deposited immunoglobulin was IgM thereafter IgG and rarely IgA or IgD One third of the patients had deposits of fibrinogen and complement C3 and one quarter had deposits of Clq Thus 9 patients had deposits of complement C3 as well as of fibrinogen Five patients exhibited in vivo fixation of IgG ANA to the nuclei of the epidermis and vessel walls

All patients had positive IgG ON ANA and two of them also GS ANA Complement fixing ON ANA was demonstrated in all but three Thirteen

had a pos tive RA latex test wh le none had basement membrane ant bod es All those who had Clq and/or C3 depos ts n the DE zone had complement fix ng ANA often n high t tres There was no correlat on between IgG ANA titres and depos ts in the DE zone

Rl e ato d artl r t s This group consisted of 59 patients with e ther class cal of defin te RA (?1) 1 females and 8 males mean age 67 years (range ?1–84) Almost one half had typ cal granular depos ts IgM was the only immunoglobul n found n the DE zone of these patients except one pat ent who also had IgG depos ts A total of 47 pat ents had IgG ANA includ ng 19 w th GS-ANA ?5 had complement fixing ANA Forty three had a posit ve RA latex test and none had basement membrane ant bod es There was no correlation between ANA latex test and DE deposits

O ler co ect e t ss e d seases The 59 pat ents of th s group were suspected of hav ng a connecti e t ssue d sease n part cular SLE and RA and 3 had scleroderma Forty-one were females and 18 males mean age 55 years (range 14–86) Th rty two had depos ts in the DE zone and two had in vivo bound IgG ANA One of the latter two patients was suspected of having a mixed connect ve t ssue d sease w th RNA ant bod es and the other one had Sjogren s syndrome The depos ts contained predom nantly IgM and C3 Twenty nine had pos tive ANA and 19 of them f xed complement Only 6 had a pos t ve RA latex test There was no correlat on between ANA latex and deposits n the DE zone

Arter s Twelve pat ents had temporal arterit s conf rmed by b opsy of the temporal artery three had Wegener s granulomatosis and two poly arterit s nodosa Ten were females and 7 males mean age 62 years (range 18–83) Only one patient w th temporal arteritis had depos ts of the IgG class n the DE zone Five had low titre IgG ON ANA w thout complement fixat on and 3 had a pos t ve RA latex test

D g tre ent S xteen females and 4? males mean age 34 years (range 4–83) were receiv ng med cat on known to elic t n some cases drug nduced SLE but w thout clin cal s gns thereof The drugs used were proca nam de ap esol ne phenyto n and carbamazep ne About 40% of these pat ents had depos ts of IgG IgM and/or C3 but not of Clq in the DE zone Depos ts were more commonly found in elderly than young pat ents Twel e had IgG ANA and 6 complement fix ng ANA n low t tres Only two had a pos t ve RA latex

I e I d r I fect o s This group comp d I(8 n patients w th bacterial or v ral nfec ?7 females and 71 males mean age 34 years n 10 mo –84 y) Of these patients 61 had c al and 47 v ral nfect ons or sequelae thereof \ l of 57 had depos ts n the DE zone pre J n n lv of IgM far less commonly of IgG C3 (Among them 19 were suffering from infec epat t s Otherw se there was no d fference n e f equency of DE zone depos ts between bac nd ral nfect ons Twenty pat ents had IgG ANA n a low t t e as a rule w thout complement f x n p opert es T vo had a pos t ve latex test and ne pa ent w th rheumat c fever had basement memb ane nt body but a negat ve ANA as well as R A la ex test and sk n b opsy

E d c e l seases Th s group of 3? pat ents included 4 w th d abetes mell tus 5 w th thyro d d ea es ? w th pern cious anaem a and 1 w th myasthen a grav s F fteen were females and 17 males mean age 40 years (range 1–84) In all 2? had depos ts n the DE zone mainly of IgG and C3 but strangely enough not Clq S x had IgG ON ANA in a low t tre and only one had a posit ve RA latex test and no basement membrane ant bod es

Alle g d s ses W th n th s group of 19 females and 18 males mean age 39 years (range ?–80) 9 had atop c dermatit s ?1 contact dermat t s and 7 asthma Ten had depos ts n the DE zone most often of IgM and C3 Six had IgG ON ANA n a low t tre and 6 had a pos tive RA latex test wh le none had basement membrane ant bod es

Nepl rop tl In th s group there were ?0 pat ents w th renal d seases 1 hav ng glomerulonephr tis 6 pyelonephrit s and ? prote nuria of unkno n cause N ne were females and 11 males mean age 45 years (range ? 77) N ne pat ents (6 with glomerulonephrit s ? w th pyelonephritis 1 w th prote nuria) had depos ts n the DE zone F ve had IgG ON ANA 4 complement f x ng ANA and 2 a pos t ve RA latex test wh le none had basement membrane ant bod es

Mal gnant d seases Out of th s group of 11 pat ents 8 had mal gnant tumours ? had myelomatos s and 1 had leukaem a S x were females and 5 males mean age 69 years (range 6? 75) Four had depos ts in the DE zone three of them a pos t ve RA latex test and nt membrane antibod es

Ulcerative colitis This small group consisted of 3 women in the age range of 33–69 years Two had exclusively C3 deposits in the DE zone 2 had positive IgG GS ANA None had a positive RA latex test or basement membrane antibodies

Other diseases A group comprising 56 controls not suspected of any of the above diseases having e g arteriosclerotic heart disease fracture and obesity It consisted of 27 females and 29 males mean age 48 years (range 18–91) Five had deposits in the DE zone 14 had IgG ON ANA in a low titre and one had a positive RA latex test

DISCUSSION

Deposition of immunoglobulins may presumably occur as a result of various pathophysiological mechanisms

a) Deposits of immunoglobulins and complement in the form of granular aggregates along the basement membrane may indicate immune complexes precipitated in type III inflammatory reactions or passively deposited in the basement membrane (4 5 7 11 12 13 14 15 19 22 23 24 27) The deposits may differ between clinically involved and uninvolved skin Possibly they may be absent in the latter depending upon the area from which the biopsy has been taken (4 14 26) The antigen in such immune complexes may be an autoantigen e DNA as may be the case in certain connective sue diseases (1 5 6 15) or a microorganism or part of such organism e g a bacterium virus fungus or protozoa as in infectious diseases (11 19 22) or a tumour-derived antigen known to circulate as immune complexes in certain malignant diseases (25) Furthermore a drug or a metabolite thereof may act after protein binding as an immunogen eliciting specific antibody production and the occurrence of immune complexes This may explain deposits observed in patients on medication (17) Another cause of deposits in the DE zone is believed to be related to an increased plasma concentration of one or more immunoglobulins leading to passive deposition of these proteins in the basement membrane (4) Patients with connective tissue diseases exhibit increased concentration of immunoglobulins so that such passive deposition is conceivable

b) Destruction of the basement membrane of non immunological nature entailing passive oozing and deposition of proteins is likewise conceivable (4)

c) Finally deposition of immunoglobulin and complement may be observed as a consequence of binding of specific antibasement membrane autoantibodies to basement membrane antigens in the skin (4 5 14 15) A characteristic of this type of reaction is linear fluorescence along the basement membrane itself thereby differing morphologically from the above mentioned granular fluorescence It represents immunological type II mechanisms as known from bullous pemphigoid in which antibasement membrane antibodies are demonstrable in serum (4 5 11 14) None of our patients had bullous pemphigoid and only one patient with rheumatic fever exhibited circulating basement membrane antibodies which however were not bound in vivo

In patients with SLE with deposits in the DE zone elution experiments have been done in order to determine the specificity of the eluted antibodies It has been claimed that these antibodies might be ANA and/or basement membrane antibodies (6 15) These findings indicate a mixed genesis of the deposits in this particular disease

Deposits of IgM were the most common immunoglobulins in practically all of our patient groups The only definite exception was observed in the diabetes mellitus group in which IgG deposits were more common than IgM In all patients the deposits were of granular appearance a finding in agreement with the fact that antibasement membrane antibody was observed in the serum of only one patient Indeed it is known that in a number of the diseases studied circulating immune complexes may occur with varying frequency and there is every likelihood that the deposits found in the majority of our positive biopsies did represent immune complex deposits (12 14) Previously we have found a correlation between immunoglobulin deposits in the skin and cryoglobulin in the serum in RA patients the latter presumably representing cold insoluble immune complexes (12)

The present results afford only a snapshot of situations in the various disease groups Longitudinal studies of individual patients might possibly disclose that the deposits in the skin occur as transient phenomena and in relation to the presence of immune complexes in the blood Such deposits of immune complex are known to take place in a number of the physiological filters of the body such

as the renal glomeruli the choroid plexus the oral and respiratory tract mucosae and the skin (3 6 13 15 19) Some authors claim to have demonstrated parallelism between deposits in the skin and kidneys (7 15 27)

Deposits of IgA and IgD were very seldom observed and deposits of IgE never Clq deposits occurred in only a few cases in other groups than that with connective tissue diseases whereas C3 and fibrinogen deposits were common in this as well as in other groups The absence of DE zone deposits in arteritides may support the view that these diseases are rarely immune complex induced and this accords with the literature (2) The finding of deposits in the skin of controls is unexplained but might possibly be due to subclinical disease (24)

Apart from studying deposits in the DE zone a number of authors have assessed biopsies with a view to deposits in the walls of arterioles and venules However this carries some uncertainty in deciding whether the fluorescent material is in an intraluminal or intraparietal situation It has not been elucidated whether the deposits in the DE zone are factors in the common atrophy of the skin in RA and other diseases

In vivo binding of ANA to epidermal and endothelial nuclei was observed in 7 patients 5 of whom were suffering from classical SLE 1 of Sjogren s syndrome and 1 was suspected of having a mixed connective tissue disease All had high titres of IgG ON ANA and the ANA fixed complement This is in agreement with the findings of Shu et al (23)

CONCLUSION

After the great initial optimism concerning the diagnostic specificity of DE zone deposits for SLE recent studies on other groups of patients have revealed that this phenomenon is common in a number of other diseases Our studies at least indicate a relationship to diseases which are often accompanied by the presence of circulating immune complexes in the blood They also show that in an ordinary series of patients with medical diseases a predominance of granular deposits must be expected These deposits may be due to deposited immune complexes or to passively deposited plasma proteins presumably in manifest diseases as well as in some subclinical conditions If the investigation could be extended by identification of antigen in the deposits such as cell components mi… organisms tumour derived antigens or d… the determination would gain a great deal in d… stic value The presence of linear deposits … the basement membrane presumably has … specificity for certain skin diseases such as … pemphigoid so that the technique is of … interest in dermatological diagnosis in which … ed skin too can be studied

ACKNOWLEDGEMENT

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REFERENCES

1 Aarden L A Immunology of DNA Thesis Rodopi 1977
2 Alarcon Segovia D The necrotizing vasculitides a new pathogenetic classification Med Clin North Am 61 241 1977
3 Atkins C J Kondon J J Jr Quismorio F P & Friou G J The choroid plexus in systemic lupus erythematosus Ann Intern Med 76 65 1972
4 Baart de la Faille Kuyer E H Lupus erythematosus An immunohistochemical and clinical study of 485 patients Thesis Utrecht 1969
5 Beutner E H Chorzelski T P Bean S & Jordon R E Immunopathology of the skin labeled antibody studies Dowden Hutchinson & Ross Stroudsburg 1973
6 Brentjens J Ossi E Albini B Sepulveda M Kano K Sheffer J Vasilion P Marine E Baliah T Jockin H & Andres G Disseminated immune deposits in lupus erythematosus Arthritis Rheum 20 962 1977
7 Burnham T K & Fine G The immunofluorescent band test for lupus erythematosus Arch Dermatol 103 24 1971
8 Burnham T K Neblett T R & Fine G The application of the fluorescent antibody technic to the investigation of lupus erythematosus and various dermatoses J Invest Dermatol 41 451 1963
9 Cohen A S Reynolds W E Franklin E C Kulka J P Ropes M W Shulman L E & Wallace S L Preliminary criteria for the classification of systemic lupus erythematosus Bull Rheum Dis 21 643 1971
10 Cormane R H Bound" globulin in the skin of patients with chronic discoid lupus erythematosus and systemic lupus erythematosus Lancet 1 534 1964
11 Cormane R H & Asghar S S Diagnostic procedures in immunodermatology J Invest Dermatol 67 129 1976
12 Donde R Permin H Juhl F Wiik A Hansen N E & Andersen R B Immune deposits in the dermo-epidermal junction in rheumatoid arthritis Scand J Rheumatol 6 57 1977

13 Gilliam J N & Cheatum D E Immunoglobulins in the larynx in systemic lupus erythematosus Arch Dermatol 108 696 1973
14 Jablonska S Beutner E H Michel B Cormane R H Holubar K Bean S F Blaszczyk M Ploem J & Saikia N K Uses for immunofluorescence tests of skin and sera Arch Dermatol 111 371 1975
15 Landry M & Sams W M Jr Systemic lupus erythematosus Studies of the antibodies bound to skin J Clin Invest 52 1871 1973
16 Larsson O Studies of small vessels in patients with diabetes Acta Med Scand (Suppl) 480 1970
17 Permin H & Sestoft L Deposits of plasma proteins in the skin during treatment with carbamazepine and diphenylhydantoin Acta Med Scand 202 113 1977
18 Permin H Wiik A Juhl F & Faber V The diagnostic value of titering antinuclear factors in the serum Ugeskr Laeger 139 2613 1977
19 Pertschuk L P Cook A W Gupta J K Broome J D Vuletin J C Kim D S Brigati D J Rainford D J & Nidsgorski F Jejunal immunopathology in amyotrophic lateral sclerosis and multiple sclerosis Identification of viral antigens by immunofluorescence Lancet 1 1119 1977
20 Quismorio F P Rea T H Levan N E & Friou G J Immunoglobulin deposits in lepromatous leprosy skin Arch Dermatol 111 331 1975
21 Ropes M W Bennet G A Cobb S Jacox R & Jessar P A 1958 revision of diagnostic criteria for rheumatoid arthritis Bull Rheum Dis 9 175 1958
22 Schiøtz P O Høiby N Juhl F Permin H Nielsen H & Svehag S E Immune complexes in cystic fibrosis Acta Pathol Microbiol Scand (C) 85 57 1977
23 Shu S Provost T Croxdale M B Reichlin M & Beutner E H Nuclear deposits of immunoglobulins in skin of patients with systemic lupus erythematosus Clin Exp Immunol 27 238 1977
24 Sutherland J C Markham R V Jr & Mardiney M R Jr Subclinical immune complexes in the glomeruli of kidneys postmortem Am J Med 57 536 1974
25 Theofilopoulus A N Andrews B S Urist M M Morton D L & Dixon F J The nature of immune complexes in human cancer sera J Immunol 119 657 1977
26 Ullman S Halberg P & Wolf Jurgensen P Deposits of immunoglobulins and complement C3 in clinically normal skin of patients with lupus erythematosus Acta Derm Venereol 55 109 1975
27 Wertheimer D & Barland P Clinical significance of immune deposits in the skin in SLE Arthritis Rheum 19 1249 1976
28 Wierzchowiecki M O Quismorio F P & Friou G J Immunoglobulin deposits in skin in systemic lupus erythematosus Arthritis Rheum 18 77 1975
29 Wiik A Jensen E & Friis J Granulocyte specific antinuclear factors in synovial fluids and sera from patients with rheumatoid arthritis Ann Rheum Dis 33 515 1974
30 Wiik A & Munthe E Restrictions among heavy and light chain determinants of granulocyte specific antinuclear factors Immunology 23 53 1972
31 — Complement fixing granulocyte specific antinuclear factors in neutropenic cases of rheumatoid arthritis Immunology 36 1127 1974

The Evolution of Asymptomatic Monoclonal Gammopathies

A Follow up of 20 Case ... of 3–14 Years

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ABSTRACT Twenty cases of "asymptomatic" monoclonal gammopathies were detected by routine electrophoresis in patient's sera or in blood donors and were followed over 3–14 years Four cases have shown a malignant evolution—two evolved toward Waldenstrom s macroglobulinemia after 3 years and two could be classified as myeloma 3 and 7 years respectively, after detection of the monoclonal protein The remaining cases were still "asymptomatic" 4 years later (7 cases), 7–9 years later (8 cases) and 14 years later (1 case) A malignant evolution occurred in approximately 20% of cases

Key words monoclonal gammopathies idiopathic monoclonal gammopathies benign essential gammopathies

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Monoclonal gammopathies (MG) can be divided into two main groups malignant and asymptomatic MG

Malignant MG includes myeloma Waldenstrom s macroglobulinemia heavy chain disease and other lymphoproliferative disorders with a typical clinical picture associated with B lymphocyte line proliferations

Asymptomatic MG has the same immunochemical abnormalities without any of the clinical features observed in malignant MG (8 9 10)

With regard to the prognosis and treatment it is very important to distinguish between malignant and asymptomatic MG If the malignant origin represents the most likely evolution for a MG asymptomatic MG has a 6% incidence in our personal experience based on 972 cases The benign nature of an asymptomatic MG is sometimes difficult to ascertain and requires a long term follow up However some criteria allow one to assume an asymptomatic MG absence of any clinical feature normal hematological and radiological findings normal or very slightly increased ESR low M protein concentration (below 15 mg/ml) absence of decrease in serum immunoglobulins and absence of Bence Jones protein (BJP) in urine We present here the results from 20 subjects followed over periods of 3–14 years

METHODS

Serum protein investigations were performed by several standard techniques The typing of the M protein was carried out by immunoelectrophoresis and the M protein quantitation by serum protein electrophoresis on cellulose acetate strip followed by densitometry The quantitative estimation of serum immunoglobulin levels was performed by radial immunodiffusion and the BJP detection by cellulose acetate electrophoresis on 50-fold concentrated urines

The following clinical and hematological investigations were performed ESR (mm/1st h) hematocrit (Hct %) hemoglobin (Hb g/100 ml) red blood cell (RBC) count leucocyte (WBC) and platelet quantitation differential count of leucocytes and in some cases bone marrow examination

CASE REPORTS

Twenty cases of asymptomatic MG were examined 6 of which were detected by routine serum protein electrophoresis in patients and the other 14 in systematic screening of blood donors (6) and followed over 3–14 years

Cases of asymptomatic MG detected in patients

Case 1 is a 59 year-old male In 1964 an IgGK asymptomatic MG was detected without BJP in urine The IgG level was 20 mg/ml (IgGK 15 mg/ml) IgM level normal and IgA slightly decreased (1 mg/ml) *Fourteen years later* the patient was still healthy without any clinical or hematological sign of myelomatosis (Hb 14 5 g/100 ml

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Abbreviations MG=monoclonal gammopathy BJP=Bence Jones protein ESR=erythrocyte sedimentation rate Hb hemoglobin Hct=hematocrit RBC=red blood cells WBC=leucocytes

13 Gilliam J N & Cheatum D E Immunoglobulins in the larynx in systemic lupus erythematosus Arch Dermatol 108 696 1973
14 Jablonska S Beutner E H Michel B Cormane, R H Holubar K Bean S F Blaszczyk M Ploem J & Sarkia, N K Uses for immunofluorescence tests of skin and sera Arch Dermatol 111 371 1975
15 Landry M & Sams W M Jr Systemic lupus erythematosus. Studies of the antibodies bound to skin. J Clin Invest 52 1871 1973
16 Larsson O Studies of small vessels in patients with diabetes Acta Med Scand (Suppl) 480 1970
17 Permin H & Sestoft L. Deposits of plasma proteins in the skin during treatment with carbamazepine and diphenylhydantoin Acta Med Scand 202 113 1977
18 Permin, H Wiik A Juhl F & Faber V The diagnostic value of titering antinuclear factors in the serum Ugeskr Laeger 139 2613 1977
19 Pertschuk L P Cook A W Gupta, J K, Broome J D Vuletin, J C Kim D S Brigati, D J Rainford, D J & Nidsgorski F Jejunal immunopathology in amyotrophic lateral sclerosis and multiple sclerosis. Identification of viral antigens by immunofluorescence Lancet 1 1119 1977
20 Quismorio F P Rea T H Levan, N E & Friou G J Immunoglobulin deposits in lepromatous leprosy skin Arch Dermatol 111 331 1975
21 Ropes, M W Bennet, G A Cobb S, Jacox R & Jessar P A 1958 revision of diagnostic criteria for rheumatoid arthritis Bull Rheum Dis 9 175 1958
22 Schiøtz, P O Høiby N, Juhl, F Permin, H, Nielsen H & Svehag, S E Immune complexes in cystic fibrosis Acta Pathol Microbiol Scand (C) 85 57 1977
23 Shu, S Provost T Croxdale, M B, Reichlin, M & Beutner E H Nuclear deposits of immunoglobulins in skin of patients with systemic lupus erythematosus Clin Exp Immunol 27 238 1977
24 Sutherland J C Markham, R V Jr & Mardiney M, R, Jr Subclinical immune complexes in the glomeruli of kidneys postmortem Am J Med 57 536, 1974
25 Theofilopoulus A N, Andrews, B S, Urist, M M Morton D L & Dixon, F J, The nature of immune complexes in human cancer sera. J Immunol 119 657 1977
26 Ullman, S Halberg, P & Wolf Jurgensen, P Deposits of immunoglobulins and complement C3 in clinically normal skin of patients with lupus erythematosus Acta Derm Venereol 55 109 1975
27 Wertheimer D & Barland, P, Clinical significance of immune deposits in the skin in SLE. Arthritis Rheum 19 1249 1976
28 Wierzchowiecki M, O Quismorio F P & Friou G J, Immunoglobulin deposits in skin in systemic lupus erythematosus Arthritis Rheum 18 77 1975
29 Wiik, A Jensen E & Friis, J Granulocyte-specific antinuclear factors in synovial fluids and sera from patients with rheumatoid arthritis Ann Rheum Dis 33 515 1974
30 Wiik, A & Munthe E, Restrictions among heavy and light chain determinants of granulocyte-specific antinuclear factors Immunology 23 53 1972
31 — Complement fixing granulocyte-specific antinuclear factors in neutropenic cases of rheumatoid arthritis. Immunology 36. 1127 1974

munoglobulin levels were normal In 1973 the IgMK M protein had disappeared ad seven years after the first examination a normal immunoglobulin level (IgG 14 IgA 2 4 IgM 1 6 mg/ml) without M-component was observed

Cases 15–20 detected as asymptomatic MG in 1971 (6) were unchanged five years later but have not been reexamined since 1975

RESULTS

Out of 20 cases classified as asymptomatic MG when first detected only four have shown a malignant evolution two presented a symptomatic Waldenstrom s macroglobulinemia after three years and two a multiple myeloma three and seven years respectively after their first examination

The other 16 cases were still asymptomatic MG four years later ($n=7$) 7–9 years later ($n=8$) or 14 years later ($n=1$) The frequency of malignant evolution is approximately 20% of cases in which asymptomatic MG are detected whereas 80% remain asymptomatic for long periods (8 cases were unchanged 7 8 or 14 years after their first detection) The length of the follow up time is of course decisive

DISCUSSION

The existence of asymptomatic MG is confirmed by several observations In 1964 Waldenstrom (9) reported cases in which the monoclonal component level remained constant for periods up to 9 years without clinical evidence of myeloma In a Swedish population 39 cases of asymptomatic MG detected by Axelsson et al in 1966 (2) and reviewed 5½ years later (3 4) have the same concentration of the monoclonal protein as at the first examination Eleven years later of the 27 subjects in this series only 3 had shown a malignant process two cases of myeloma and one case of malignant lymphoma (1) In the report by Despont et al (5) one case classified as asymptomatic remained unchanged 13 years after detection However in 7 out of 39 cases the presence of bone marrow infiltration of plasma cells or lymphocytes suggests a malignant evolution The occurrence of a typical myeloma in the course of asymptomatic MG many years after discovery of the M protein is unusual although Kyle and Bayrd (7) have reported a case without clinical evidence of myeloma for 16 years but showing a typical myeloma 18 years after the first examination

The present study confirms the existence of non malignant asymptomatic MG Most of our patients show no malignant process more than five years after the first examination and do not require treatment The low percentage (about 20%) of asymptomatic MG followed by malignant evolution allows one to conclude that the detection of an "asymptomatic MG in a patient s serum is not synonymous with a malignant process but should lead to repeated examinations of the patient over long periods A non malignant transformation occurring after the first four or five years is a good argument for a prognosis of non malignant lymphoproliferative disorder

REFERENCES

1 Axelsson U An eleven year follow-up on 64 subjects with M-components Acta Med Scand 201 173 1977
2 Axelsson U Bachmann U & Hallén J Frequency of pathological proteins (M-components) in 6995 sera from an adult population Acta Med Scand 179 235 1966
3 Axelsson U & Hallen J Review of fifty four subjects with monoclonal gammopathy Br J Haematol 15 417 1968
4 — A population study on monoclonal gammopathy Acta Med Scand 191 111 1972
5 Despont J P Fluckiger R Ghaith A Hausser E Jeannet M Monnier J Scheidegger J J Siegenthaler P Wettstein P & Cruchaud A Idiopathic paraproteinemia Helv Med Acta 34 401 1969
6 Fine J M Lambin P & Leroux P Frequency of monoclonal gammopathy (M components) in 13400 sera from blood donors Vox Sang 23 336 1972
7 Kyle R A & Bayrd E D "Benign" monoclonal gammopathy a potentially malignant condition? Am J Med 40 426 1966
8 Ritzmann S E Loukas D Sakai H Daniels J C & Levin W C Idiopathic (asymptomatic) monoclonal gammopathies Arch Intern Med 135 95 1975
9 Waldenström J The occurrence of benign essential monoclonal (M type) non-macromolecular hyperglobulinaemia and its differential diagnosis IV Studies in the gammopathies Acta Med Scand 176 345 1964
10 — Monoclonal and polyclonal hypergammaglobulinaemia Cambridge University Press London 1968

A Patient with Cronkhite-Canada Syndrome, Myxedema and Muscle Atrophy

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ABSTRACT A case of Cronkhite-Canada syndrome is presented The patient had alopecia, onychodystrophy and gastrointestinal polyposis mainly in the stomach and duodenum, with transient diarrhea and hypoproteinemia Marked atrophy and weakness of the shoulder girdle muscles due to myopathy were also present In addition she had primary hypothyroidism The outcome of the disease is usually fatal within months, but so far our patient is alive four years after the onset of symptoms The pathological changes, pathophysiology, symptoms course and treatment of this rare disorder of unknown etiology are discussed

Key words Cronkhite Canada syndrome gastrointestinal polyposis myopathy myxedema

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In 1955 Chronkhite and Canada described a syndrome with generalized gastrointestinal polyposis and ectodermal changes with hair loss nail atrophy and skin pigmentation (4) Subsequently about 50 cases have been reported To our knowledge the patient reported here is the first case of Cronkhite Canada syndrome reported from Norway and the third in Scandinavia The syndrome should be distinguished from others with gastrointestinal polyposis such as Peutz Jeghers syndrome familial colonic polyposis Gardner s syndrome juvenile polyposis and Turcot s syndrome

CASE REPORT

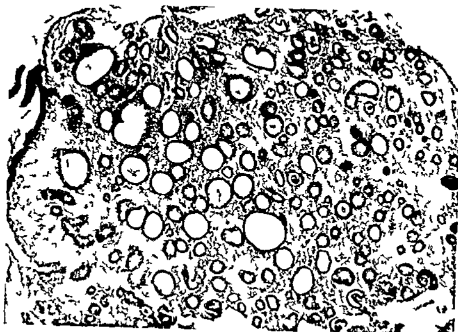
The patient is a 66-year-old woman first admitted to the Department of Medicine in Aug 1974 She then complained of diarrhea and weight loss of 11 kg over the last six months She also had noticed loss of finger and toenails and scalp hair There was no family history of gastrointestinal disorders On admission she was thin with scanty scalp hair The finger and toenails had fallen off and partly regrown There was moderate edema of both legs

Laboratory investigations ESR was 10 mm/h Hb 12 6 g/100 ml and serum sodium potassium chloride calcium phosphate magnesium cholesterol vitamin B_{12} and folic acid were all normal Renal and liver function tests were normal Serum albumin was low 27 g/l T_4 iodine was below normal 36 nmol/l TSH above normal 45 0 μU/ml and no thyroid antibodies were found Serum CK was normal A pentagastrin test showed gastric anacidity and a secretin test demonstrated normal pancreatic bicarbonate secretion Three-day stool collection showed normal amounts of fat and no blood The urine was normal

Radiography of the gastrointestinal tract showed multiple polypoid filling defects mainly in the stomach and duodenum Fiberendoscopic examination of the upper gastrointestinal tract revealed a normal esophagus and the stomach and duodenum were full of small smooth sessile polyps A barium enema showed only a few filling defects in the colon and coloscopy demonstrated two small polyps in the sigmoid and one in the transverse colon The radiological changes in this patient have been extensively described by Maurer et al (16)

Histological examination of the gastric mucosal biopsies showed atrophic superficial and deep glands with some intestinal metaplasia There was excessive edematous lamina propria which was infiltrated with eosinophils lymphocytes histiocytes and plasma cells Many of the glands showed cystic dilatation with mucous retention The appearances were characteristic of the Cronkhite Canada syndrome (Figs 1 and 2)

The patient was admitted to the Department of Neurology in July 1975 after several years with pains in the shoulders and arms and recently also proximal weakness in the upper limbs Neurological examination showed symmetrical severe atrophy and weakness of the shoulder girdle muscles with less involvement of the distal muscles of the upper limbs The achilles reflex was absent bilaterally The other myotatic reflexes were weak but symmetrical Sensation was normal There was no affection of the lower extremities or of the cranial nerves Radiological examination showed degenerative changes and kyphosis of the cervical spine There was subluxation of the shoulder joints and arthrography showed large irregular joint spaces Cervical myelography with Amipaque® showed



F g 1 Gastr c mucosa show ng d lated cyst c g ands and edematous lam na prop a Hema oxyl n and eos n ×54

normal sp nal canal with no s gns of nerve root compres on The cerebrosp nal flu d and the Queckenstedt test were bo h normal Electromyography of various arm and leg muscles revealed myopath c changes Motor nerve conduct on eloc ty and d stal delay n the left peroneal and ulnar nerves were normal B ops es of the t b al s anterior and delto d muscles showed sl ght myopath c changes

The pat ent has rece ed no treatment apart from thyrox ne sod um The d arrhea mp o ed spontaneously Her na ls and ha r are almost normal The neurological and elect omyograph c f nd ngs we e unchanged when the ent was reexam ned n March 1976

DISCUSSION

The et ology of the Cronkh te Canada syndrome s unknown It usually affects m ddle aged and elderly males and females but has been reported n a 31 year old man (23)

The polyps are generally found throughout the d gestive tract By studying the reports of 45 cases Tokuyasu et al (24) found that the polyps were localized in the esophagus in 2 cases (7 10) stomach in 41 small intestine in 36 colon n 41 and



F g 2 Gastric mucosa show ing atrophy of superf c al glands and edema of lam na propria Hema oxyl n and eos n ×54

rectum in *30* cases Our patient had polyps mainly in the stomach and duodenum

Originally the polyps were described as adenomatous (4 6 9 15 20) In 1971 Canada Diner (2) reviewed the two cases from 1955 in the light of the subsequent cases reported She found that the intestinal lesions of the syndrome usually had the characteristics of cystically dilated hyperplastic glands Therefore she proposed that they should be classified as inflammatory pseudopolyps or cystic glandular dilatation rather than true neoplasms or adenomatous polyps The view that the polyps are regenerative and not neoplastic is supported by the fact that they may regress (8 10 13 14 25) Intestinal malignancy has however been described in a few cases (1 6 12 22) Whether this represents malignant change in a polyp or is coincidental is unknown

The characteristic ectodermal changes of the Cronkhite Canada syndrome are alopecia onychodystrophy and skin pigmentation Our patient had scanty scalp hair and transient onychodystrophy but no skin pigmentation It is unlikely that these anomalies are due to nutritional deficiencies as they may precede diarrhea by several months and also because other protein losing enteropathies do not cause similar changes

As demonstrated by our patient the main symptom of the disease is diarrhea which may be accompanied by steatorrhea Possible causes of the diarrhea have been discussed by Johnson et al (8) The gastrointestinal loss of fluid may lead to low serum electrolytes followed by muscular weakness or tetanic manifestations Our patient had normal serum electrolytes but she had hypoproteinemia which is a common finding in this kind of patients Using isotopic techniques protein leakage into the gastrointestinal tract has been demonstrated (1 7 8 18 21 23 25) and it has been postulated that it takes place through the dilated glands (*19*)

Some patients like ours improve either spontaneously or following symptomatic and supportive treatment (5 10 12 21 25) Other patients deteriorate progressively or improve temporarily before they die in a state of cachexia due to uncontrolled loss of protein and electrolytes (1 4 6 7 8 9 13 18 19 22 24) The course of the disease probably depends on the extent of the gastrointestinal polyposis Our patient is still alive and in reasonable health four years after the onset of symptoms This is not exceptional and still longer survivals have been reported (3 5 19)

A trial with antibiotics has been recommended by Johnson et al (8) but this has not shown any clear effect except in one patient (5) who also had jejunal diverticulosis Corticosteroids have been claimed to give dramatic recoveries in two cases (3 23) but in others there has been no or only a temporary effect (9 19) Anabolic steroids have also been tried alone (20) or combined with surgery (19) with good results To prevent the protein loss partial gastrectomy (3 11 14 15) or colectomy (7 19 23 26) have been carried out and remissions have been seen following these procedures (11 14 15 19 26)

Our patient also had myxedema and myopathy The radiological findings in the shoulders might be suggestive of neuropathic joint changes Myxedema has previously been reported in a patient with the Cronkhite Canada syndrome (1) but this seems to be a coincidental finding Patients with myxedema may have myopathy but this is usually reversible after treatment with thyroxine (17) The severe atrophy and weakness of the shoulder girdle muscles in our patient did not improve However a relation between these two disorders cannot be excluded Since cervical myelography was normal it was very unlikely that cervical nerve root compression could be the cause of the atrophy and paresis Cotterill et al (3) reported a patient with Cronkhite Canada syndrome who subsequently developed muscle atrophy in the extremities but who also had a sensory deficit increased tendon reflexes and extensor plantar reflexes The apparent cause was a cervical myelopathy Diffuse muscle atrophy has been noticed in three other cases (6 7 11) and in one of them the wasting was particularly evident in the shoulder regions (11) These findings suggest that the myopathic changes of our patient may be part of the Cronkhite Canada syndrome

REFERENCES

1 Burnell R H Cronkhite-Canada syndrome Med J Aust 1 347 1976
2 Canada Diner W The Cronkhite-Canada syndrome Radiology 105 715 1971
3 Cotterill J A Day J L Hughes J P Paulley J W & Turk E The Cronkhite-Canada sy Postgrad Med J 49 .68 1973
4 Cronkhite L. W & Canada W J C gastrointestinal polyposis An unusual syr

polyposis pigmentation alopecia and onychotrophia N Engl J Med 252 1011 1955
5 Cunliffe W J & Anderson J Case of Cronkhite Canada syndrome with associated jejunal diverticulosis Br Med J 4 601 1967
6 Da Cruz G M G Generalized gastrointestinal polyposis An unusual syndrome of adenomatous polyps alopecia onychorotrophia Am J Gastroenterol 47 504 1967
7 Jarnum S & Jensen H Diffuse gastrointestinal polyposis with ectodermal changes Gastroenterology 50 107 1966
8 Johnson G K Soergel K H Hensley G T Dodds W J & Hogan W J Cronkhite-Canada syndrome Gastrointestinal pathophysiology and morphology Gastroenterology 63 140 1972
9 Johnston M M Vosburgh J W Wiens A T & Walsh G C Gastrointestinal polyposis associated with alopecia pigmentation and atrophy of the fingernails and toenails Ann Intern Med 56 935 1962
10 Kennedy J A & Hirson C A transient syndrome with Peutz Jeghers features and ectodermal change Proc R Soc Med 54 234 1961
11 Kindblom L G Angervall L Santesson B & Selander S Cronkhite-Canada syndrome Cancer 39 2651 1977
12 Koehler P R Kyaw M M & Fenlon J W Diffuse gastrointestinal polyposis with ectodermal changes Radiology 103 589 1972
13 Krause H Cronkhite-Canada Syndrom Med Welt 23 508 1972
14 Libner G Vinz L & Essbach U Beitrag zum Cronkhite-Canada Syndrom Z Gesamte Inn Med 29 76 1974
15 Manousos O & Webster U Diffuse gastrointestinal polyposis with ectodermal changes Gut 7 375 1966
Maurer H J Hansen A & Maurer B Cronkhite Canada Syndrom Roentgendiagnostik Fortschr Geb Roentgenstr Nuklearmed 122 399 1975
17 McArdle B Metabolic and endocrine myopathies In Disorders of voluntary muscle (ed J N Walton) pp 749-750 Churchill Edinburgh and London 1974
18 Mielke F W Diffuse Polyposis ventriculi Polyposis intestini—Cronkhite Canada Syndrom Z Gastroenterol 11 529 1973
19 Miyoshi M Fujii H Iwasa N Nishitani T Nishimura S Inatomi I Matsumoto H & Katake K Two autopsy cases of diffuse gastrointestinal polyposis with ectodermal changes Am J Gastroenterol 64 357 1975
20 Nishiyama S Mori S & Harada S Gastrointestinale Polyposis mit universeller Alopecie Onychodystrophie und Pigmentation der Haut Arch Klin Exp Derm 221 144 1965
21 Orimo H Fujita T Yoshikawa M Takemoto T Matsuo Y & Nakao K Gastrointestinal polyposis with protein losing enteropathy abnormal skin pigmentation and loss of hair and nails (Cronkhite Canada syndrome) Am J Med 47 445 1969
22 Shibuya C An autopsy case of Cronkhite-Canada's syndrome—generalized gastrointestinal polyposis pigmentation alopecia and onychotrophia Acta Pathol Jpn 22 171 1972
23 Takahata J Okubo K Komeda T Kono T & Fukui I Generalized gastrointestinal polyposis associated with ectodermal changes and protein losing enteropathy with a dramatic response to prednisolone Digestion 5 153 1972
24 Tokuyasu K Takebayashi S Takahara O & Uchiyama E An autopsy case of Cronkhite-Canada syndrome Gastroenterol Jpn 11 215 1976
25 Witzel L Classen M Roesch W & Demling L Cronkhite Canada Syndrom Dtsch Med Wochenschr 96 989 1971
26 Zdansky E & Riederer J Gastrointestinale Polypose Adenomatose mit Hypoproteinamie und ektodermale Storungen Radiol Clin 32 254 1963

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Trauma and Hodgkin's Disease

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ABSTRACT In two patients, Hodgkin's disease developed apparently after a trauma The relationship between trauma and Hodgkin's disease is discussed The trauma may be the initiating factor in the development of the pathological process, or it may be a localizing factor in a patient already suffering from Hodgkin's disease As long as these questions remain unanswered and a causal relationship cannot be excluded, it will be reasonable, if accident insurance problems are involved, to give the patient the benefit of the doubt

Key words Hodgkin s disease trauma

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It has occasionally been postulated that Hodgkin s disease may develop after severe traumatic lesions The question is not just of theoretical interest as insurance problems may come into the picture The possibility that Hodgkin s disease may develop after a severe traumatic lesion was discussed by Begemann and Kaboth in their excellent treatise on Hodgkin s disease (2) and the considerations presented below are mainly based on their views It must at once be emphasized that even though there are traumatic tissue lesions and the resultant histological changes appear in the lymph nodes draining the region concerned this does not say anything about the aetiology of the lymphogranulomatosis (e g virus infection or tumour) Begemann and Kaboth stated that almost all doctors who have been involved in the treatment of patients suffering from Hodgkin s disease have observed cases in which the disease has developed in direct relation to infections or severe traumatic lesions (see also Klima and Gott (7) who cite further references) It is however not possible to draw the conclusion that a causal relationship always exists in such cases For example Chevallier and Bernard (6) regarded them as due to mere coincidence

It is however impossible to refrain from suspecting a causal relationship in some cases in which the trauma the subsequent tissue alterations and the lymphogranulomatosis involve the same region (1 3)

If a relationship between a trauma and the development of Hodgkin s disease is to be considered the traumatic tissue lesions must persist after the trauma and an unquestionable histological picture of Hodgkin s disease must be found in a regional lymph node It is perhaps also permissible to take a causal relationship into consideration in cases in which traumatic tissue lesions are complicated by a severe chronic infection which in turn sensitizes the organism so that a distant affection of the antibody producing reticulolymphocytic tissue should be considered The infections and the traumatic tissue lesions which may lead to Hodgkin s disease are as a rule severe and protracted However a number of cases are on record in which a relatively slight trauma has apparently been the factor initiating the lymphogranulomatosis It goes without saying that such an assumption is justified only in cases in which the time interval between the trauma and the development of Hodgkin s disease is short and especially when the first manifestation of Hodgkin s disease occurs in lymph nodes which are primarily affected only in rare cases

Begemann and Kaboth (2) saw two cases in which inoculations were apparently the exciting factor Cases of postvaccinal lymphadenitis have also been reported by Bichel (5) Begemann and Kaboth concluded their discussion on the development of Hodgkin s disease after severe infections and traumatic tissue lesions by stating that since a variety of factors may be involved in the initiation of the lymphogranulomatous process it seems a

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polyposis pigmentation alopecia and onychotrophia N Engl J Med 252 1011 1955
5 Cunliffe W J & Anderson J Case of Cronkhite Canada syndrome with associated jejunal diverticulosis Br Med J 4 601 1967
6 Da Cruz G M G Generalized gastrointestinal polyposis An unusual syndrome of adenomatous polyps alopecia onychorotrophia Am J Gastroenterol 47 504 1967
7 Jarnum S & Jensen H Diffuse gastrointestinal polyposis with ectodermal changes Gastroenterology 50 107 1966
8 Johnson G K Soergel K H Hensley G T Dodds W J & Hogan W J Cronkhite Canada syndrome Gastrointestinal pathophysiology and morphology Gastroenterology 63 140 1972
9 Johnston M M Vosburgh J W Wiens A T & Walsh G C Gastrointestinal polyposis associated with alopecia pigmentation and atrophy of the fingernails and toenails Ann Intern Med 56 935 1962
10 Kennedy J A & Hirson C A transient syndrome with Peutz Jeghers features and ectodermal change Proc R Soc Med 54 234 1961
11 Kindblom L G Angervall L Santesson B & Selander S Cronkhite Canada syndrome Cancer 39 2651 1977
12 Koehler P R Kyaw M M & Fenlon J W Diffuse gastrointestinal polyposis with ectodermal changes Radiology 103 589 1972
13 Krause H Cronkhite Canada Syndrom Med Welt 23 508 1972
14 Libner G Vinz L & Essbach U Beitrag zum Cronkhite-Canada Syndrom Z Gesamte Inn Med 29 76 1974
15 Manousos O & Webster U Diffuse gastrointestinal polyposis with ectodermal changes Gut 7 375 1966
Maurer H J Hansen A & Maurer B Cronkhite Canada Syndrom Roentgendiagnostik Fortschr Geb Roentgenstr Nuklearmed 122 399 1975
17 McArdle B Metabolic and endocrine myopathies In Disorders of voluntary muscle (ed J N Walton) pp 749–750 Churchill Edinburgh and London 1974
18 Mielke F W Diffuse Polyposis ventriculi Polyposis intestini—Cronkhite Canada Syndrom Z Gastroenterol 11 529 1973
19 Miyoshi, M Fujii H Iwasa N Nishitani T Nishimura S Inatomi I Matsumoto H & Katake K Two autopsy cases of diffuse gastrointestinal polyposis with ectodermal changes Am J Gastroenterol 64 357 1975
20 Nishiyama S Mori S & Harada S Gastrointestinale Polyposis mit universeller Alopecie Onychodystrophie und Pigmentation der Haut Arch Klin Exp Derm 221 144 1965
21 Orimo H Fujita T Yoshikawa M Takemoto T Matsuo Y & Nakao K Gastrointestinal polyposis with protein losing enteropathy abnormal skin pigmentation and loss of hair and nails (Cronkhite Canada syndrome) Am J Med 47 445 1969
22 Shibuya C An autopsy case of Cronkhite Canada's syndrome—generalized gastrointestinal polyposis pigmentation alopecia and onychotrophia Acta Pathol Jpn 22 171 1972
23 Takahata J Okubo K Komeda T Kono T & Fukui I Generalized gastrointestinal polyposis associated with ectodermal changes and protein losing enteropathy with a dramatic response to prednisolone Digestion 5 153 1972
24 Tokuyasu K Takebayashi S Takahara, O & Uchiyama E An autopsy case of Cronkhite-Canada syndrome Gastroenterol Jpn 11 215 1976
25 Witzel L Classen M Roesch W & Demling L Cronkhite Canada Syndrom Dtsch Med Wochenschr 96 989 1971
26 Zdansky E & Riederer J Gastrointestinale polypose Adenomatose mit Hypoproteinamie und ektodermale Storungen Radiol Clin 32 254 1963

REFERENCES

1 Begemann H Klinische und experimentelle Beobachtungen am immunisierten Lymphknoten Schulz Freiburg 1953

2 Begemann H & Kaboth W In Begemann H Handbuch der inneren Medizin Zweiter Band Blut und Blutkrankheiten Funfte Auflage Teil 5 Krankheiten des Lymphocytaren Systems pp 1_0–121 Springer Verlag Berlin Heidelberg and New York 1974

3 Begemann H & Merker H In Arbeit und Gesundheit H 70 94 Thieme Stuttgart 1959

4 Bi hel J Folia Clin Int (Barc) 22 2 1972

5 — Acta Med Scand 199 523 1976

6 Chevallier P & Bernard J La maladie de Hodgkin Masson et Cie Paris 1932

7 Klima R & Gott E In Handbuch des gesamten Hematologie Funfter Band pp 125–126 Urban & Schwarzenberg Munchen and Berlin 1964

International advances in surgical oncology vol 1 edited by G P Murphy 288 pages US $ 36 Liss Inc New York 1978

This is a valuable book for anyone working in the field of oncology McBride and coworkers review 20 years experience with regional chemotherapy by isolation perfusion About 900 melanoma patients have been treated In most cases melphalan has been used but combinations of drugs have also been tried The perfusion can be combined with hyperthermia which probably potentiates the action of cytotoxic drugs No controlled clinical studies are available but used prophylactically in combination with local excision in melanoma and soft tissue sarcoma the overall survival rate is better than for patients treated with surgery alone Adjuvant immunotherapy is the subject of an excellent critical review by Goodnight and Morton Different types of immunotherapy (non specific active specific and passive immunotherapy) are described It seems to be important to have a minimal tumor burden and to have the immunostimulant administered in an optimal way McKneally s randomized trial with intrapleural administration of BCG after operation of early lung carcinoma may serve as an illustration Accumulated data on malignant melanoma have possibly shown some beneficial effects The combination of immunotherapy and cytostatic treatment is discussed

Sufrin and coworkers present data showing different levels of adenosine deaminase in lymphocytes from patients with renal and transition cell carcinoma and they believe this reflects differences in immune mechanisms in these diseases Holyoke and Goldrosen summarize work on colon cancer and carcinoembryonic antigen levels (CEA) cellular immune competence and the tumor specific immunity Initial CEA levels may have some role in risk staging and detection of recurrences Dermal antigen testing T cell counts and lymphoblastogenesis dies do not seem to be useful in this disease The thors present further data on tumor specific immunity hich may prove useful in clinical practice Veronesi and collaborators give an extensive review on malignant melanoma They summarize work on epidemiology diagnosis and treatment The different histological types of malignant melanoma are discussed and discussions on classification and nomenclature are reviewed The TNM classification of WHO Collaborating Center for Evaluation on Methods of Diagnosis and Treatment of Melanoma is presented Data on the prognostic aid of Clark s levels of invasion and Breslow s measurement of tumor thickness are summarized The paper is a very good presentation of current melanoma problems

Wells and coworkers review the malignant phenotypes in familial polyposis Several of these syndromes seem to overlap instead of being well defined entities Sakakibara and coworkers have analyzed recurrences of early gastric cancer in relation to sex age diameter location and macroscopic type of the tumor microscopic lymph node metastasis invasion of lymphatic vessels and veins in the gastric wall histological type and infiltrative growth Kinochita and coworkers present data on long term survivors after surgical treatment of upper and mid thoracic esophageal cancer The authors provide data which suggest that the histological malignancy is of great importance The volume ends with a summary on laser and surgical oncology Different types of lasers are presented and the application of laser treatment of different types of cancers is described

The volume is highly recommended especially the review of melanoma problems different aspects of which are carefully described in three of the chapters

Ulrik Ringborg Stockholm Sweden

The infection prone hospital patient Edited by J F Burke and G Y Hildick Smith 252 pp $16 50 Little Brown & Co Boston 1978

The book contains 16 presentations from a symposium designed to bring together leading physicians and investigators to provide a thorough consideration of the current state of research and its clinical application to the management of infection prone hospital patients The book covers basal aspects on neutrophils macrophages and lymphocytes It provides clinical considerations on host defense mechanism defects in children It also discusses preventive measures for infections in patients with acute leukemia after kidney transplantation and major surgery

The book tries to unite theoretical and practical considerations in the compromised host This is done best in the chapters on host defense mechanism defects in children The chapters on the role of cellular function recapitulate our present knowledge in this field The clinical part focuses on practical preventive measures One presentation deals with the possible role of transfer factor The implications of interferon are not discussed Various trials to increase the cell mediated immune response by treatment with immunostimulating drugs are not mentioned

The book is interesting to read and provides references for further reading Its scientific information is of a high standard and the various presentations smoothly complement one another For the specialist in the field most data are already known For ordinary clinicians the information given on theoretical subjects will hardly affect daily work The value of the book is in presenting clinicians with theoretical data and laboratory investigators with clinical data that will stimulate further research

Stig Cronberg Malmo Sweden

Acta Med Scand 205 353 360 1979

Evaluation of Indices of Alcohol Intake in a Population of 60-Year-Old Men in Uppsala, Sweden

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ABSTRACT The following indices of alcohol intake were evaluated in a health survey of 60-year-old men in Uppsala medical history of alcohol intake on the day prior to screening presence of alcohol in urine (U alcohol) on the day after screening registration at the Temperance Board in the community For 28% of the participants one or two of the indices pointed to casual or long standing habits of alcohol intake Eighteen per cent considered themselves to be teetotallers S triglycerides S-cholesterol B glucose S urate and S gammaglutamyl transferase (S GT) concentrations were analysed No significant differ ences in S-l pid and B glucose concentrations were found between the alcohol index groups and a control group The mean values of S-urate and S GT how ever were significantly higher in the alcohol index groups and proved to be reasonably discriminatory between them and the control group in that 63% of the subjects who had S-GT values of ≥0 70 μkat/l had one or two indices of alcohol intake The cor responding figure for subjects who had S GT values of <0 30 μkat/l was 22% U alcohol was found in 36 subjects (11%) 14 of whom also had other indices of alcohol intake the remaining 22 probably represent ed examples of casual alcohol intake It is concluded that determinations of U alcohol S GT and S urate concentrations could be of diagnostic value in unclear cases in which increased alcohol intake is suspected of being the etiological factor of a disease or a symptom

Key words alcohol intake γ glutamyl transferase alcohol in ur ne

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Assessment of alcohol intake n a population s d f fcult Th s is probably one reason why alcohol intake has not been invest gated in most population studies Several population stud es have been per formed to elucidate which risk factors increase the risk of ischem c heart disease (IHD) The main such factors proposed are smoking high blood pressure (BP) elevated lipid concentrations and impa red glucose tolerance (16 36 57 61) As a h gh alcohol intake may cause obesity increased serum l pid concentrations high BP and impaired glucose toler ance studies of alcohol intake are valuable when assessing risk factors for IHD

This study concerns methodological aspects of four parameters that are being used to evaluate the alcohol intake in a populat on of 60-year old men in Uppsala These four parameters are determi nations of urinary alcohol (U alcohol) and serum gammaglutamyl transferase (S GT) concentrations history of alcohol intake prior to screening and registrat on f any at the Temperance Board

STUDY POPULATION AND METHODS

S bjects

The screen ng p ocedures have been descr bed in deta l elsewhere (58) The total population comprised all men born n 1915 who were living in Uppsala n 1975 They numbered 4?? and were all inv ted to a health survey n the autumn of 1975 an inv tat on that was accepted by 331 men g v ng a part c pat on rate of 78 4%

A letter was sent to the subjects one week n advance request ng them to fast and not to smoke in the 8–10 hours preced ng the exam nat on The inves gat ons were per formed in the morn ng on weekdays Mondays through Fridays On average f ve subjects were nvest gated each day

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A prel m nary report was given at the Annual General Meeting of the Swed sh Soc ety of Med cal Sc ences (Lakaresallskapets R ksstamma) 19 6

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Abbrev a ns IHD ischem c heart disease BP blood pressure SBP = systol c BP DBP d astol c BP ECG = electrocard ogram GT = gammaglutamyl transfera e G gluco e Tg trigl ycer des Chol cholesterol U = urinary B = blood S = serum f fast ng

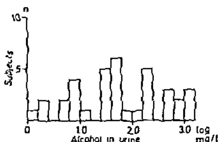


Fig 1 Distribution of logarithmically converted U alcohol concentrations in group 2 (n=36) Log x=1 75 anti log x~56 8

Information was obtained from the records of the Temperance Board in the municipality concerning the men who were registered there No attempts were made however to evaluate the severity of their alcohol abuse from these records

Medical history

This was obtained by means of a self administered questionnaire modified after Collen et al (11) together with a personal interview In one question the subject was asked if he had taken any alcoholic drinks in the foregoing 24 hours The personal interview included questions on intake of medicines occurrence of diseases smoking habits and marital status A specific question of stress behavior was included and read Do you feel impatient when held up in traffik while driving or when talking to a tedious person when you are in a hurry? In the personal interview the subject was also asked if he was a teetotaller defined as abstention from all beverages containing more than 1 8% of alcohol

Physical examination

BP was measured after 10 min of rest in the recumbent position using a mercury wall manometer (Kifa Ercameter) with a 12 cm wide and 35 cm long rubber bladder Systolic and diastolic BP (SBP DBP) were read to the nearest 5 mmHg mark The DBP was measured at the disappearance of the Korotkoff sounds (phase 5) The radial pulse was counted after 10 min rest immediately prior to the BP measurement Height and weight in under shorts were measured The heights and weights of the 50-year old men in the same community (28) were used as a reference to define the relative body weight

Laboratory investigations

Blood samples were taken in the fasting state from an antecubital vein for automated determination of blood glucose (fB-G) (30) serum triglycerides (fS-Tg) (35) serum cholesterol (fS-Chol) (40) S GT (56) and serum urate (S urate) (13) concentrations

S GT concentration was determined in 325 men Analysis was not performed due to hemolysis of the sample in one case in two cases pronounced lipemia made S-GT analysis impossible finally three samples were lost One of these subjects reported an alcohol intake in the 24 hours preceding screening

The subjects were carefully instructed to save all urine for 24 hours following the screening A two-litre plastic bottle with a screw top containing no antibacterial substances was supplied for the purpose The reason for the urine collection was not mentioned to the subjects Urine samples were obtained from 327 of the 331 participants Two collections failed because of mental incapacity one subject refused for reasons of work and one who was registered at the Temperance Board did not return with his sample The latter subject was the only alcohol index case (see below) among those who failed to collect 24-hour urine The volume of urine voided was measured next day and 200 mg sodium iodide was added The samples were then stored at 4°C until the survey had been completed whereupon analyses were carried out

Electrocardiogram (ECG)

A 12 lead ECG comprising the standard leads I II and III unipolar leads aVR aVL and aVF and leads V_1-V_6 was recorded in all participants The ECG recordings were interpreted independently by two physicians at the Department of Clinical Physiology using the criteria of Goldmann (23) and the Minnesota Code (52)

Statistical methods

Conventional statistical methods were used to calculate the mean values and standard deviations (S D) When testing the significances of differences between mean values Student s t test (two-tailed) was used The χ^2 test was used when comparing frequencies

RESULTS

There were four groups with the following indices of alcohol intake *Group 1* comprised 47 men (14%) who gave an affirmative reply to the question about alcohol intake in the 24 hours preceding the screening In *group 2* there were 36 men (11%) in whom U-alcohol was found on the day after screening

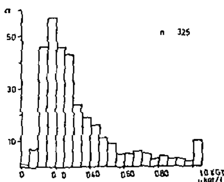


Fig 2 Distribution of S-GT concentrations among the whole screened population of 60-year old men (n=325)

Table I *fB G fS Chol S urate (mean ± S D and range) S GT and fS Tg (antilog of the mean and range) in the alcohol index groups and the control groups*

	Control group A	Control group B	Control group C	Group 1	Group 2	Group 3	Group 4
S-GT (µkat/l)	0 24 0 09–1 61	0 27 0 10–2 34	0 26 0 12–2 34	0 38 0 11–1 65	0 32 0 09–1 19	0 33 0 10–1 34	0 47 0 13–1 34
fB G (mmol/l)	5 3±1 3 3 8–13 8	5 8±2 0 4 2–15 2	5 2±0 9 4 2–5 9	5 4±1 4 4 1–13 9	5 5±1 8 4 3–14 7	5 8±1 8 3 9–11 0	5 3±0 8 4 5–7 4
fS Chol (mmol/l)	6 1±1 1 3 9–9 9	6 1±1 2 4 0–10 0	5 7±0 9 4 3–7 4	6 2±1 1 4 0–9 7	6 3±1 2 3 3–8 4	6 2±1 2 3 3–8 7	6 3±1 1 3 3–7 6
fS-Tg (mmol/l)	1 51 0 52–5 90	1 62 0 38–7 80	1 48 0 38–5 60	1 38 0 55–3 70	1 51 0 50–5 60	1 48 0 50–7 80	1 41 0 50–3 60
S urate (µmol/l)	270 7±63 7 130 9–470 1	256 9±55 4 131 1–345 7	254 5±53 6 131 1–327 8	297 5±83 9 172 6–553 4	301 7±83 3 130 9–553 4	265 4±52 4 142 8–446 3	334 4±96 4 232 1–553 4

(mean concentration 252 mg/l) Ten of them reported an alcohol intake on the day preceding the screening and a further four were registered at the Temperance Board The distribution of U alcohol concentrations was skewed to the right Logarithmically transformed values for this group are shown in Fig 1 *Group 3* comprised 25 men (8%) who were registered at the Temperance Board The quite low figure for Temperance Board registration compared with data reported by another author (31) might be explained by the high figure 19% for non participants *Group 4* consisted of 15 men (5%) in whom a combination of two of the above mentioned findings was noted

Ninety three subjects (28%) had one or two indices of casual or long standing alcohol intake A group of 93 randomly selected subjects from participants in the same health survey was formed and will be referred to as *control group A* These subjects were chosen as the person following an alcohol index case in the screening programme and did not belong to any of the groups described above Sixty subjects (18%) considered themselves to be teetotallers They will be referred to as *control group B* It included a subgroup of 24 subjects who denied diseases and intake of medicines and is called *control group C*

S GT levels in relation to indices of alcohol intake

The distribution of S GT concentrations in the population (n=325) is shown in Fig 2 The distribution was skewed to the right The mean value was 0 26 µkat/l (range 0 01–2 34) calculated on logarithmic transformed data The mean values of S-GT concentrations in the four groups with indices of alcohol intake were 0 38 0 32 0 33 and 0 47 µkat/l respectively (Table I) all higher than the mean values of control group A

The relative and absolute distributions of the subjects of the four alcohol index groups within the range of S-GT concentration are shown in Fig 3 It is inferred from this figure that a proportionally larger number of men with high than with low S GT concentrations belonged to one of these groups Thus of the subjects with a S GT concentration of ≥0 70 µkat/l (n=30) 63% were allocated to an alcohol index group The corresponding figure for those with S GT concentrations of <0 30 µkat/l (n=200) was 22% In addition the subjects belong

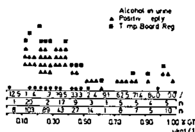


Fig 3 Distribution of S-GT concentrations among the whole screened population of 60-year old men (n=325) and in relation to the alcohol index groups (n=93) The first row indicates the relative number the second the absolute number of men who were alcohol index cases within the intervals of S-GT concentrations and the third the total number of subjects within the intervals of S-GT concentrations

Table II *Comparison between mean values of log S-GT S urate and fS Chol in the three control groups and the four alcohol index groups*

	Control groups	Group 1	Group 2	Group 3	Group 4
S-GT (μkat/l)					
	A	$p<0.001$	$p<0.05$	$p<0.05$	$p<0.001$
	B	$p<0.05$	n s	n s	n s
	C	$p<0.05$	n s	n s	$p<0.001$
S-urate (μmol/l)					
	A	n s	$p<0.05$	n s	$p<0.05$
	B	$p<0.01$	$p<0.01$	n s	$p<0.01$
	C	$p<0.01$	$p<0.01$	n s	$p<0.01$
fS Chol (mmol/l)					
	A	n s	n s	n s	n s
	B	n s	n s	n s	n s
	C	$p<0.05$	$p<0.05$	n s	n s

n s = not significant

ing to the index groups were evenly distributed over the whole range of S GT concentrations This figure also shows that some subjects with a S-GT concentration of ≥0 70 μkat/l exhibited two indices of alcohol intake

There were 55 subjects (17%) with an S GT level of >0 50 μkat/l which value is considered by Laurell et al (39) to be the upper reference limit for this enzyme Twenty three (42%) of these subjects had one or two indices of alcohol intake The remaining 32 subjects were investigated for the occurrence of diseases and drug treatment Nineteen subjects reported known diseases the most common being diabetes mellitus and essential hypertension Atrial fibrillation chronic bronchitis and ulcerative colitis were reported by occasional subjects None of the men reported drug treatment that might be associated with increased S-GT concentrations (51) No disease or drug treatment were found in 13 subjects

U alcohol in relation to the other indices of alcohol intake

As mentioned above among the group of 36 men in whom U alcohol was found on the day after screening 14 had indices of alcohol intake besides the urinary findings These 14 men had a mean S GT value of 0 43 μkat/l and a mean U alcohol concentration of 387 mg/l In the other 22 subjects in this group the corresponding figures were 0 28 μkat/l and 192 mg/l No correlations between U alcohol and S GT were found in the whole group or in the two subgroups mentioned above

fB G fasting serum lipids and S urate in relation to indices of alcohol intake

No significant differences were found when the mean fS Tg or fB G values of the alcohol index groups were compared with the corresponding values of the three control groups The mean fS Chol values in groups 1 and 2 were significantly higher ($p<0.05$) than the mean fS-Chol value of control group C The mean S urate levels of all alcohol index groups except group 3 were significantly higher ($p<0.01$) than the mean S urate levels of control groups B and C The differences are shown in Table II

Prevalence of high BP and diabetes mellitus in the alcohol index groups control groups A and B and in the entire screened population

The prevalence of hypertension defined as a resting DBP of ≥105 mmHg or ongoing treatment for hypertension was 19% in the whole screened population (n=331) in control group A (n=93) and in group 1 Nine of the 36 subjects in group 2 had hypertension according to the same criteria One third of the men in group 4 were hypertensive

The prevalence of diabetes mellitus—defined as fB G >6 6 mmol/l or ongoing treatment with a diet insulin and/or oral antidiabetic drugs—was not significantly different in the index groups compared with control groups A and B In the whole screened population the prevalence of diabetes mellitus was 4 5%

Table III *Pathological ECGs (%) and number of subjects with codable abnormalities in the alcohol index groups and in the control groups*

One subject may occur more than once

	Control group A ($n=93$)	Control group B ($n=60$)	Control group C ($n=24$)	Group 1 ($n=47$)	Group 2 ($n=36$)	Group 3 ($n=25$)	Group 4 ($n=15$)
Codable items							
I Q wave items	2	2				1	
II QRS axis deviation	5	5	3	2	2		1
III High QRS amplitude	7	11	6	2	2		1
IV S-T wave changes	4	3		1	1	1	
V T wave items	8	4		9	3	1	3
VI AV conduction defects	1	2	1				
VII Ventricular conduction defects	3	1			1	1	
VIII Rhythm	3	3	3	4	1		
IX Miscellaneous	3	3	3	1			
Pathological ECGs (%)	19	12	4	28	11	8	20

Smoking habits, marital status, and stress behavior in the alcohol index groups and the control groups

The largest proportion of smokers, two-thirds of the men, was found in group 4. This frequency and that in group 3 were both significantly higher ($p<0.05$) than in control group A. Only 15% of the subjects in control group B were smokers, which was significantly less ($p<0.001$) than the number of smokers in all four alcohol index groups.

There were significantly more ($p<0.01$) unmarried men in group 3 than in control group A.

The number of men who replied affirmatively to the specific question on stress behavior mentioned above were consistently higher in all four alcohol index groups than in the control groups. The differences, however, were significant only in group 2 ($p<0.05$) and group 4 ($p<0.01$) compared to control group A.

BP, pulse rate, and relative body weight in relation to the alcohol index groups and to the control groups

The mean SBP and DBP values of control group A were 144.5±24.2 and 86.1±12.1 mmHg, respectively. The mean pulse rate was 65.6±9.7 beats/min and the mean relative body weight was 1.01±0.13. No differences were found between these values and those of the alcohol index groups, except for the higher ($p<0.05$) mean pulse rate, 70.1±11.3 beats/min, in group 3.

ECG findings in the alcohol index groups and in the control groups

The ECG findings coded according to the Goldmann criteria (23) and to the Minnesota Code (52) are shown in Table III. Pathological resting ECGs were found in 28% of the men in group 1. The most dominant finding in this group according to the Minnesota Code was T wave items, which also held for control group A. The number of men with rhythm disorders in group 1 is noteworthy: two of them had atrial fibrillation. Another case of atrial fibrillation was found in group 2.

DISCUSSION

The use and abuse of alcohol have been studied for both medical and social reasons (10, 44, 55). Many authors have tried to classify alcohol dependency according to the amounts ingested and the frequency of alcohol consumption. The best known classifications are given by Jellinek (34). Many subjects, however, have a common tendency to underestimate their drinking habits when giving their medical history. Securing a valid history of alcohol intake is often time consuming.

The definition of alcohol dependency varies, but in many studies approximately 10% of the population above 15 years of age are classified as addicts or chronic alcoholics. Prevalence studies in Sweden by Hagnell and Tunving (26), Inghe (32), and Alstrom (3) have yielded this figure. Similar figures are reported by Fox (19) and Edwards (14) in the

US A common finding is that a large proportion of the alcoholics are unknown to the medical authorities (15 25) We found that 10% of the population were registered at the Temperance Board 11% had U alcohol on the day after screening Altogether 28% of all subjects presented with one or two indications of casual or long standing alcohol intake

The aim of this study was to find means of objectively evaluating alcohol intake Findings of elevated S-GT concentrations have been mentioned by some authors (37 38 49 50 53) as one way of diagnosing long standing alcohol consumption causing dysfunction of the liver The enzyme GT is distributed throughout the body Thus there are reports of elevated S-GT values in diabetes mellitus IHD elevated BP chronic obstructive lung disease and hepatic disorders of other causes than excessive consumption of alcohol (4 5 29 33) Phenytoin and barbiturates have been found to raise S-GT levels through enzyme induction (51)

In our study all alcohol index groups had significantly higher mean S-GT values than control group A In the whole screened population ($n=331$) 30 (63%) of the subjects with an S-GT level of ≥0 70 μkat/l but only 200 (22%) of those with an S-GT level of <0 30 μkat/l were alcohol index cases A finding of an S-GT concentration of ≥0 70 μkat/l as judged from this study should be suggestive of alcohol intake as a probable cause

Determinations of U alcohol as was done in our study might add further information in unclear cases However as mentioned by Weinig et al (60) there are obvious difficulties in relating the amounts of alcohol in urine to the amounts ingested Bonnichsen (7) suggested that 3–4% of alcohol ingested is excreted in urine as ethyl alcohol

The mean BP levels did not differ between the alcohol index groups and the control groups Shah (54) has reported higher BP levels in alcohol addicts than in teetotallers The mean body weight indices of the alcohol index groups did not differ significantly from those of the control groups in our study

ECG findings of T wave items and rhythm disorders were noted in group 1 admitting alcohol intake on the day prior to screening ECG changes in connection with alcohol intake have been discussed by other investigators (2 20 27) The pathophysiological basis for the ECG changes is unknown and is probably not related to alcohol itself but rather to the metabolites produced by the oxidation of alcohol as discussed by several authors (17 18 24 47 48) many referring to alcoholic cardiomyopathy

The effect of alcohol on carbohydrate metabolism is two-fold Thus an excessive alcohol intake on a single occasion might result in hypoglycemia especially if the supplies of glycogen are inadequate However long term alcohol intake often leads to glucose intolerance and diabetes mellitus (6 12 46) The highest prevalence figure for diabetes mellitus among the various alcohol index groups of the 60-year old men in Uppsala was 8% in the group of men registered at the Temperance Board

The mean serum lipid levels did not differ significantly between the alcohol index groups and the control groups except for the mean fS Chol values of groups 1 and 2 compared to control group C ($p<0.05$) Some authors (1 59) have reported a relationship between findings of hyperlipidemia and alcohol consumption However subjects with a defective lipid clearance mechanism (9 22 43) seem most prone to develop hyperlipidemia on intake of alcohol The mean S-urate levels were significantly higher in our groups 2 and 4 than in the control groups B and C Hyperuricemia as a result of high alcohol intake has been reported by several authors (21 41 45)

Those who attended this health examination certainly represented alcohol consumers similar to those in large parts of the general population The non-participants probably had more severe drinking habits to judge from the large proportion of men registered at the Temperance Board in this group Registration at the Temperance Board often implies severe alcohol dependency The register also comprises persons who have been convicted due to alcohol intoxication in traffic Routines vary between physicians in the reporting of alcoholics to the Temperance Board and one must also consider the possibility that a subject may remain registered for a long time after a possible cessation of alcohol dependency The addition of other parameters besides Temperance Board registration and medical history might add information in cases where increased alcohol intake is suspected Such cases might be therapeutic failures diabetes mellitus hyperlipidemia cardiac arrhythmias of unclear origin and the multisymptomatic patient presenting with various complaints such as low back pains headache gastritis and insomnia

Our results suggest that repeated determinations of S GT S urate and U alcohol concentrations might be of further diagnostic value U alcohol per se in connection with increased S-GT values and S urate values must be judged as strong evidence of increased alcohol intake

ACKNOWLEDGEMENT

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REFERENCES

1 Adelson S E & Keys A Diet and some health characteristics of 123 business and professional men Nutr Rev 21 294 1963

2 Alexander C S Idiopathic heart disease I Analysis of 100 cases with special reference to chronic alcoholism Am J Med 41 213 1966

3 Alstrom C H Alkoholproblemet och samhallet 20 års medicinsk forskning Norstedts Stockholm 1965

4 Aronsen K F Nosslin B & Phil B The value of gammaglutamyltranspeptidase as a screen test for liver tumour Acta Chir Scand 136 17 1970

5 Barton A D Powers J L & Lourenco R V Gamma glutamyltranspeptidase in chronic obstructive pulmonary disease Proc Soc Exp Biol Med 146 99 1974

6 Bedo M S Balint I & Zsinka A T Zuckerbelastungsuntersuchungen an Alkoholikern Dtsch Z Verdau Stoffwechselkr 23 29 1963

7 Bonnichsen R K Personal communication 1976

8 Bonnichsen R K & Theorell H An enzymatic method for the microdetermination of ethanol Scand J Clin Lab Invest 3 58 1951

9 Bottiger L E Carlson L A Hultman E & Romanus V Serum lipids in alcoholics Acta Med Scand 199 357 1976

10 Carlsson C Alkoholism som sjukdom Scandinavian University Books Goteborg 1970

11 Collen M F Cutler J L Siegelaub A B & Cella R L Reliability of a self administered medical questionnaire Arch Intern Med 123 664 1969

12 Conn H O Schreiber W M Elkington S G & Johnson T R Cirrhosis and diabetes I Increased incidence of diabetes in patients with Laennecs cirrhosis Am J Dig Dis 14 837 1969

13 Domagk G F & Schlicke H H A calorimetric method using uricase and peroxidase for the determination of uric acid Anal Biochem 22 219 1968

14 Edwards G Epidemiology applied to alcoholism A review and an examination of purposes Q J Stud Alcohol 34 28 1973

15 Edwards G Hawker A Hensman C Peto J & Williamson V Alcoholics known or unknown to agencies Epidemiological studies in a London suburb Br J Psychol 123 169 1973

16 Epstein F H Glucose intolerance and cardiovascular disease Triangle 12 3 1973

17 Evans W Alcoholic cardiomyopathy Am Heart J 61 556 1961

18 — Alcoholic myocardiopathy Progr Cardiovasc Dis 7 151 1964

19 Fox R A multidisciplinary approach to the treatment of alcoholism Am J Psychiatry 123 769 1967

20 Fredriksen P & Hed R Clinical studies in chronic alcoholism III Cardiac changes in chronic alcoholism Acta Med Scand 162 203 1958

21 Garrod A B A treatise on gout and rheumatic gout 3rd ed Longmans & Green London 1876

22 Ginsberg H Olefsky J Farquhar J W & Reaven G M Moderate ethanol ingestion and plasma triglyceride levels A study in normal and hypertriglyceridemic persons Ann Intern Med 80 143 1974

23 Goldmann M J Principles of electrocardiography 5th ed Lange Los Altos 1964

24 Gould L Zahir M De Martino A & Gomprecht R F Cardiac effects of a cocktail JAMA 218 1799 1971

25 Hagnell O & Sandahl A Dold alkoholism på sjukhus Alkoholfrågan 2 43 1972

26 Hagnell O & Tunving K Alkoholism i en totalbefolkning Alkoholfrågan 8 293 1971

27 Hamby R I & Raia F Electrocardiographic aspects of primary myocardial disease in 60 patients Am Heart J 76 316 1968

28 Hedstrand H A study of middle aged men with particular reference to risk factors for cardiovascular disease Ups J Med Sci (Suppl) 19 1975

29 Hedworth Whitty R B Whitfield J B & Richardson R W Serum gammaglutamyltranspeptidase activity in myocardial ischemia Br Heart J 29 432 1967

30 Hjelm M & De Verdier C H A methodological study of the enzymatic determination of glucose in blood Scand J Clin Lab Invest 15 415 1963

31 Hjortzberg Nordlund H Abuse of alcohol in middle aged men in Gothenburg Acta Psychiatr Scand (Suppl) 199 1968

32 Inghe G Mental and physical illness among paupers in Stockholm Acta Psychiatr Scand (Suppl) 121 1958

33 Jacobs W L W Gammaglutamyltranspeptidase in diseases in the liver cardiovascular system and diabetes mellitus Clin Chim Acta 38 419 1972

34 Jellinek E M The disease concept of alcoholism Hillhouse Press New Haven 1960

35 Kessler G & Lederer H Fluorometric measurement of triglycerides In Automation in analytical chemistry (ed L T Skeggs) pp 341–344 Mediad New York 1965

36 Keys A Taylor H Blackburn H Brozek J Anderson T & Simonson E Mortality and coronary heart disease among men studied for 23 years Arch Intern Med 128 201 1971

37 Lamy J Baglin M C Ferrant J P & Weill J Emploi de la mesure de la gammaglutamyltranspeptidase serique pour controller le succes des cures de

desintoxication antialcoolique Clin Chim Acta 60 103 1975
38 Lamy J Baglin M C Weill J & Aron E Gammaglutamyltranspeptidase serique et alcoolisme Diagnostic et control du sevrage Nouv Presse Med 4 487 1975
39 Laurell C B Lundh B & Nosslin B Klinisk kemi i praktisk medicin Studentlitteratur Lund 1976
40 Levine J B & Zak B Automated determination of serum total cholesterol Clin Chim Acta 10 381 1964
41 Lieber C S Jones D P Losowsky M S & Davidson C S Interrelation of uric acid and ethanol metabolism in man J Clin Invest 41 1863 1962
42 Lum G & Gambino S R Serum gammaglutamyltranspeptidase activity as an indicator of disease of liver pancreas or bone Clin Chem 18 358 1972
43 Mendelsohn J H Significance of alcohol induced hypertriglyceridemia in patients with type IV hyperlipoproteinemia Ann Intern Med 80 270 1974
44 Myrhed M Alcohol consumption in relation to factors associated with IHD A co-twin study Acta Med Scand (Suppl) 567 1974
45 Olin J S Devenyi P & Weldon K L Uric acid in alcoholics Q J Stud Alcohol 34 1202 1973
46 Phillips G B & Safrit H F Alcoholic diabetes induction of glucose intolerance with alcohol JAMA 217 1513 1971
47 Regan T J Ethyl alcohol and the heart Circulation 44 957 1971
48 Regan T J Levinson G E Oldewurtel H A Frank M J Weisse A B & Moschos C B Ventricular function in non-cardiacs with alcoholic fatty liver role of ethanol in the production of cardiomyopathy J Clin Invest 48 397 1969
49 Rollason J G Pincherle G & Robinson D Serum gammaglutamyltranspeptidase in relation to alcohol consumption Clin Chim Acta 39 75 1972
Rosalki S B Rau D Lehmann D & Prentice M Determinations of serum gammaglutamyltranspeptidase activity and its clinical applications Ann Clin Biochem 7 143 1970
51 Rosalki S B Tarlow D & Rau D Plasma gammaglutamyltranspeptidase elevation in patients receiving enzyme inducing drugs Lancet 2 376 1971
52 Rose G A & Blackburn H Cardiovascular survey methods WHO Monographies vol 56 Geneva 1968
53 Rutenberg A M Goldberg J A & Pineda E P Serumgammaglutamyltranspeptidase activity in hepatobiliary pancreatic disease Gastroenterology 45 43 1963
54 Shah V V Environmental factors and hypertension With particular reference to prevalence of hypertension in alcohol addicts and teetotallers In The epidemiology of hypertension (ed J Stamler R Stamler & T Pullman) pp 204–206 Grune & Stratton New York 1967
55 Sundby P Alcoholism and mortality Universitetsforlaget Oslo 1967
56 Szasz G A kinetic photometric method for serum gammaglutamyltranspeptidase Clin Chem 15 124 1969
57 Truett J Cornfield J & Kannel W A multivariate analysis of the risk of coronary heart disease in Framingham J Chron Dis 20 511 1967
58 Waern U Health and disease at the age of 60 Findings in a health survey of 60-year-old men in Uppsala and comparisons with 10 year younger men Ups J Med Sci 83 153 1978
59 Wallgren H & Barry H Actions of alcohol part I–II Elsevier Amsterdam London and New York 1970
60 Weinig E Zink P & Reinhardt G Uber die forensiche Bedeutung der Alkoholkonzentration im Urin Blutalkohol 7 307 1970
61 Wilhelmsen L Wedel H & Tibblin G Multivariate analysis of risk factors for coronary heart disease Circulation 48 950 1973

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Zinc Supplementation in Alcoholic Cirrhosis

A Double Blind Clinical Trial

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ABSTRACT A double blind clinical trial with zinc sulfate 0 2 g three times daily, and a placebo was performed in 30 patients with biopsy proven alcoholic liver cirrhosis The disease was in a stable phase, and none of the patients showed evidence of a decompensated liver function Parameters of liver function taste acuity dark adaptation and of zinc and vitamin A metabolism were followed for six weeks In the zinc treated group of 16 patients serum zinc rose from a normal mean value of 13 3 to 17 4 μmol/l, whereas the mean serum vitamin A level remained practically unaltered within the normal range, 1 89 at the entry and 1 83 μmol/l at the end of the study Plasma prothrombin and serum alkaline phosphatase levels of the zinc group increased and serum bilirubin and serum carotene decreased significantly The dark adaptation did not change but the taste function was significantly improved during zinc treatment The results indicate that zinc supplementation causes alleviation of certain abnormalities of cirrhotics which deserves further attention

Key words alcoholic cirrhosis alkaline phosphatase dark adaptation taste function zinc sulfate therapy

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It has long been known that patients with liver cirrhosis may show subnormal levels of zinc and vitamin A in plasma and liver and paradoxically hyperzincuria (5 10 17 18) In zinc deficient rats low plasma zinc concentrations are also accompanied by low levels of vitamin A (14) As in cirrhotic man (15) the low plasma vitamin A levels of zinc deficient rats reflect low plasma concentrations of retinol binding protein which is produced by the liver (15) Following zinc therapy in zinc deficient rats plasma vitamin A increases whereas vitamin A supplementation has no such effect (14) The effect of zinc is attributed to a normalization of hepatic production of retinol binding protein which is needed for the transport of vitamin A and for its mobilization from liver stores (15)

Cirrhosis is often associated with night blindness (10) and colour blindness (2) which may be resistant to vitamin A therapy even in high doses It has been suggested that such disorders are due to zinc deficiency (3) Vallee et al (18) observed that the bromsulfalein retention test was improved in six cirrhotics who were given small oral doses of zinc As controlled trials with zinc for cirrhosis have never been performed the present investigation was undertaken with the purpose of studying the effects of oral zinc sulfate on parameters of liver function as well as of dark adaptation taste function and of vitamin A and zinc metabolism in patients suffering from alcoholic cirrhosis in a stable phase

PATIENTS AND METHODS

Thirty two outpatients with biopsy proven alcoholic cirrhosis were initially included in a 6-week double blind clinical trial with zinc sulfate and a placebo The patients were randomly assigned either to a group receiving coated zinc sulfate tablets of 0 2 g (≈45 mg zinc) or to a group receiving lactose tablets of identical appearance The tablets and the code were provided by the Pharmacy Laboratory Rigshospitalet One tablet was to be taken three times daily after meals The mean age of the females was 59 years (range 53–66) and of the males 56 years (range 30–73) All patients had a past history of alcoholism which had caused liver cirrhosis but they now claimed to abstain from alcohol Twenty six (80%) of the patients had had bleeding from oesophageal varices (10 cases) encephalopathy (10 cases) or ascites (16 cases) Sixteen patients were receiving diuretics (thiazides furosemide thalidone) together with a potassium supplement (Kaleorid®) (14 cases) or with spironolactone (Aldactone®) (2 cases) Two patients took disulfiram (Antabus®) The individual medication was continued unaltered during the trial The general health and the social condition of the patients were considered to be good and the liver disease was in a stable phase Two drop-outs were due to hospitalization at an

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Table I Laboratory analyses and taste score values of alcoholic cirrhosis patients receiving oral zinc sul[…] or placebo for 6 weeks

Mean values with range in parentheses

	Reference limits	Zinc group (n = 16)			Placebo group (n = 14)		
		Entry	6 weeks	p^b	Entry	6 weeks	p^c
Hb (g/l)	113–169	145 (121–172)	143 (113–166)	>0 2	150 (113–171)	148 (118–174)	>0
Thrombocytes (10^9/l)	160–400	157 (37–262)	152 (52–260)	>0 3	166 (80–267)	167 (68–264)	>0
P albumin (g/l)	35 4–54 1	34 5 (27 2–41 4)	34 0 (27 9–42 8)	=0 3	34 2 (26 0–43 2)	34 8 (25 7–41 7)	>0
P IgG (g/l)	6 62–15 7	12 5 (5 5–26 2)	12 7 (8 5–26 3)	>0 5	13 3 (7 9–20 7)	12 6 (7 3–19 6)	<0
P IgA[a] (g/l)	0 56–3 30	3 42 (1 20–8 09)	3 28 (1 14–7 92)	>0 6	3 88 (2 44–7 87)	3 91 (2 30–7 24)	>0
P prothrombin (arb U)	0 57–1 43	0 85 (0 50–1 45)	0 94 (0 51–1 30)	<0 05	0 76 (0 54–1 16)	0 78 (0 54–1 32)	>0
S bilirubin[a] (μmol/l)	2–17	12 9 (7–25)	10 1 (4–24)	<0 005	21 8 (9–87)	19 7 (9–105)	>0
S alkaline phosphatase (U/l)	50–275	270 (155–665)	304 (190–560)	<0 05	281 (200–500)	269 (205–400)	>0
S alanine aminotransferase[a] (U/l)	10–40	26 2 (13–110)	28 1 (13–98)	>0 4	28 6 (20–57)	27 7 (17–63)	>0
S magnesium (mmol/l)	0 78–1 03	0 76 (0 63–0 94)	0 78 (0 57–0 93)	>0 2	0 77 (0 49–0 91)	0 76 (0 56–0 94)	>0
S zinc (μmol/l)	10 6–18 9	13 3 (10 2–16 8)	17 4 (11 6–22 8)	<0 001	12 5 (8 9–18 3)	12 7 (9 5–15 7)	>0
[…]tamin A (μmol/l)	0 90–2 16	1 89 (0 76–3 57)	1 83 (0 76–3 27)	>0 5	1 70 (0 72–2 99)	1 61 (0 66–2 63)	>0
[…]ene[a] (μmol/l)	0 76–3 33	1 11 (0 15–3 79)	0 91 (0 16–2 51)	<0 005	1 05 (0 44–3 08)	1 10 (0 32–4 49)	>0
Taste score (arb U)		7 4 (2–11)	10 2 (6–12)	<0 001	7 8 (4–11)	9 0 (3–12)	>0

[a] Mean value calculated as the antilog of mean of logarithmic values
[b] Paired t test Unpaired t test

other hospital and to severe gastric pain after ingestion of zinc tablets respectively

Blood tests were performed at two- or six week intervals (Table I Fig 1) Furthermore at the entry and at the end of the trial leucocytes serum (S) protein plasma (P) sodium P potassium S creatinine and P IgM levels were estimated S zinc was determined by atomic absorption spectrophotometry S vitamin A and S carotene (α and β forms) by a photometric method (Medicinsk laboratorium A/S Copenhagen) Blood samples were drawn between 1 and 2 p m after at least 4 hours fasting

The dark adaptation was measured before and after the trial by means of the Goldmann Weeker adaptometer The absolute threshold for light perception was determined after 15 min stay in darkness without any preadaptation The scores of 200 normal military pilots tested under similar conditions were used as reference values A performance was classified as being normal borderline or abnormal

The colour vision was evaluated by Ishihara s test for colour blindness and further by the pseudoisochromatic plates (AO H R R) tritan and tetratan plates in Hard Rand and Rittler

At each visit to the clinic the taste function was determined semiquantitatively by a modification of the method described by Krarup (6) The patient was to recognize sweet salt sour and bitter by tasting increasing concentrations of sucrose (4 10 and 40%) sodium chloride (2 5 7 5 and 15%) citric acid (1 5 and 10%) and quinine chloride (0 075 0 5 and 1%) The solutions were presented in a random order except for the quinine series which always came last One drop of the solution was applied to the middle of the tongue and the patient was allowed to taste with closed mouth Three points were

Comparison of zinc and placebo group (p)	
Entry	6 weeks
>0 5	>0 4
>0 6	>0 4
>0 8	>0 6
>0 5	>0 9
>0 3	>0 2
>0 3	>0 05
<0 05	<0 02
>0 6	>0 5
>0 6	>0 9
>0 8	>0 5
>0 3	<0 001
>0 5	>0 4
>0 7	>0 4
>0 6	>0 1

scored if the lowest concentration of the test solution led to identification of the tastant two and one respectively if the middle and the highest concentration was recognized If the tastant was not identified no point was scored The sum of the scores was taken as a measure of the patient s overall taste function Smokers ($n=10$) were asked not to smoke for three hours prior to the visit to the clinic

The results were analysed statistically by means of the paired and unpaired t test Logarithmic transformation of the results was made if required so that calculations could be based on normal distributions

RESULTS

Thirty patients completed the trial When the code was broken it was found that 16 (5 females and 11 males) had received zinc sulfate and 14 (2 females and 12 males) placebo Apart from differences in the pretreatment value of S bilirubin the two groups were comparable with respect to biochemical findings (Table I) as well as to past history of liver disease Both groups had subnormal pretreatment mean values of P albumin and S magnesium and high levels of P IgG and P IgA

Only 23% of the patients (2 in the zinc and 5 in the control group) had slightly lowered S zinc concentrations which could be explained in all cases by subnormal P albumin levels S vitamin A was decreased in 10% of the patients (3 in the zinc group) whereas S carotene was subnormal in 33% (4 in the zinc and 6 in the control group)

Except for an unexplained fall in P IgG in the placebo group significant changes in the parameters studied occurred in the zinc treated patients only (Table I) The changes were found in the levels of S zinc P prothrombin S bilirubin S alkaline phosphatase and S carotene and in the taste function as depicted in Fig 1 The changes in S vitamin A were statistically insignificant (Table I) as were the changes in other tests not included in the table

The dark adaptation studies failed to reveal any beneficial effect of the zinc supplementation In the zinc group one abnormal case and 11 borderline cases (75%) were found before the trial and four abnormal and seven borderline cases (69%) after the trial In the control group nine (64%) had borderline values before the trial and four abnormal and six borderline values (71%) after the trial The difference between the number of abnormal and borderline cases in the zinc group and in the control group and the difference between the changes in the groups were statistically insignificant ($p>0\ 5$)

Red/green colour blindness was present before and after the trial in four out of the 23 males which is a little more than could be expected but inconclusive due to the small population examined No change in the colour vision was observed during the study especially not in the yellow/blue component

Three patients in the zinc group experienced a marked improvement of their well being within the first month of zinc medication Their taste score values increased from 7 to 11 9 to 11 and 5 to 11 respectively They noticed spontaneously the improved taste function One of them a male of 68 years experienced smell sensations for the first time for many years S zinc S vitamin A and S carotene of the patients were essentially normal

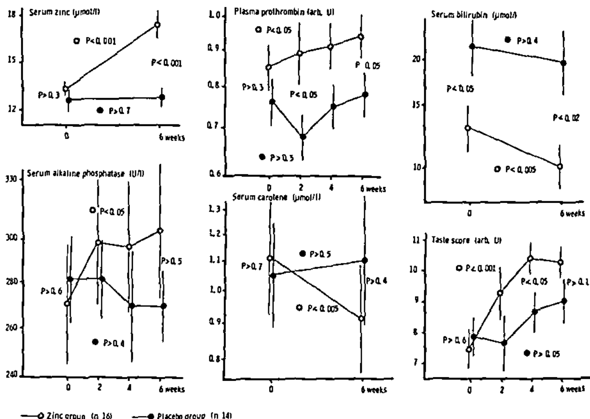


Fig 1 Comparison of patients receiving zinc supplementation and patients receiving a placebo Single circles (open and closed) indicate that the *p* value is calculated by means of paired *t* test of the results of each group before and after six weeks of treatment Otherwise the *p* value indicates comparison between the groups by unpaired *t* test Vertical lines denote S E M Prothrombin bilirubin alkaline phosphatase and carotene levels were not normally distributed In these cases the ordinates are logarithmic and the mean is calculated as the antilog of the mean of logarithmic values Likewise the S E M is based on the logarithmic values

Seven of the 16 patients receiving zinc sulfate complained of transient uneasiness and/or gastric pain shortly after ingestion of the tablets The discomfort gradually ceased as the medication was continued except in two patients who suffered from recurrent episodes of diarrhoea throughout the trial Three patients in the placebo group had slight gastric oppression following tablet ingestion

DISCUSSION

The present results verify that zinc therapy has some effects in stable alcoholic cirrhosis As expected S zinc of the zinc treated group rose significantly during the trial and a rise was also seen in the S alkaline phosphatase activity The two parameters run a parallel course in the hereditary zinc deficiency disorder acrodermatitis enteropathica (19) in conditional zinc deficiency due to long term zinc free parenteral nutrition (20) and in chronic zinc deficiency of beer drinkers with chronic alcoholism and malabsorption (21) Simkin (12) who gave oral zinc sulfate to rheumatoid arthritics also noted a rise in the S alkaline phosphatase activity of his patients Determinations of alkaline phosphatase isoenzymes were not performed systematically in that or in the present investigation So it is still not known whether the fluctuations primarily occurred in liver type bone type or intestinal type alkaline phosphatase The mechanism of action of this zinc effect is unknown but it is probably relevant that the alkaline phosphatase enzyme as isolated from Escherichia coli (11) is a zinc metalloenzyme As the phenomenon has been reported only in disease states connected with zinc deficiency it can perhaps be taken as evidence of latent zinc deficiency in our patients In this study it seems unlikely that the rise in S alkaline phospha

tase activity was related to cholestasis as at the same time S bilirubin decreased significantly in the zinc treated group A toxic effect of zinc sulfate on the liver cells seems unlikely and the mean level of S alanine aminotransferase also remained unaltered within the reference limits

Weser et al (23) have shown that rat liver RNA polymerase and the synthesis of liver RNA and protein are stimulated by supplemental zinc If this holds true for humans too it might explain the significant increase in the prothrombin level of the zinc treated patients but not the fact that the P albumin concentration of the patients remained unaltered low during six weeks of zinc supplementation Six weeks of therapy however may be too short to evaluate such an effect of zinc It was studied because in various mammalian species zinc deficiency produces alterations in albumin and gamma globulin plasma levels (7 9) not unlike those found in cirrhosis of man

We were unable to confirm any beneficial effect of zinc on the dark adaptation of cirrhotics as recently reported by Morrison et al (8) This might be due to the relatively good vitamin A and zinc nutrition of our patients as evaluated from the normal pretreatment serum levels of these elements It might also be related to the examination method used which particularly with untrained persons is influenced by a large variability that conceals minor changes in the adaptation function

The normal S vitamin A level of the zinc treated patients remained unchanged during the trial whereas a marked fall was observed in the S carotene level Morrison et al (8) reported normal S carotene but highly decreased S vitamin A levels in cirrhotics with significantly lowered S zinc values The low S vitamin A levels reported probably reflected a decreased concentration of plasma retinol binding protein as often found in cirrhosis (13) but it might also be related to a disturbed conversion of carotene to vitamin A Further studies will clearly be needed to clarify the possible role of zinc in the interconversion of carotene to vitamin A It is well known that zinc is involved in the metabolism of vitamin A Alcohol dehydrogenase is a zinc metalloenzyme (16) which in combination with NAD (cozymase) also catalyzes the conversion of vitamin A or retinol to retinal which combined with opsin constitutes rhodopsin Retinal oxidase which converts vitamin A alcohol to retinoic acid in the tissues is another zinc dependent enzyme (16) Furthermore zinc seems to play an important role in the synthesis by the liver of retinol binding protein (15) It is not known whether zinc is specifically involved in the synthesis of opsin in the retina

Cirrhosis is often associated with abnormalities of the smell and taste functions (1) A relation to abnormalities in zinc copper and vitamin A metabolism has been proposed but is still unclear The positive response to zinc supplementation in the present study suggests that zinc is involved but other factors may play a role too Since idiopathic loss of taste is correctable by zinc copper and nickel supplements (4) the improvement of the patients taste function by zinc cannot be taken as evidence of pre-existing zinc deficiency

Kahn et al (5) reported that the degree of aberrant zinc metabolism in cirrhosis seems to be correlated to the severity of liver dysfunction All cirrhotics of the present study were in a stable phase and none showed evidence of a decompensated liver function This probably explains their overall normal S zinc values This however does not exclude low zinc levels in the tissues and in diseased organs such as the cirrhotic liver Beneficial effects of the zinc supplementation on some liver parameters were observed and it might be speculated that severely affected cirrhotics especially those with a high alcohol intake would benefit more from zinc therapy Clinically overt zinc deficiency has been reported in cirrhotics mainly living on alcohol and beer which happen to be extremely poor in zinc (21 22) In such cases zinc has an impressive effect on zinc related skin symptoms such as widespread eczema craquele dermatitis on the acral parts of the extremities (acrodermatitis) and in the anogenital region as well as on the poor hair growth (21 22) The present results suggest that zinc also has positive effects in cirrhotics without such evidence of zinc deficiency

The question of routine administration of zinc in alcoholic cirrhosis must await clarification by further research

ACKNOWLEDGEMENT

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REFERENCES

1 Burch R E Sackin D A Jetton M M & Sullivan J F Decreased acuity of taste and smell in cirrhosis Gastroenterology 67 A-4 781 1974

2 Cruz Coke R Colour blindness and cirrhosis of the liver Lancet 1 1113 1965
3 Halsted J A & Smith J C Night blindness and chronic liver disease Gastroenterology 67 193 1974
4 Henkin R I & Bradley D F Hypogeusia corrected by Ni^{+} and Zn^{++} Life Sci 9 701 1970
5 Kahn A M Helwig H L Redeker A G & Reynolds T B Urine and serum zinc abnormalities in disease of liver Am J Clin Pathol 44 426 1965
6 Krarup B Kliniske smagsundersøgelser Store Nordiske Videnskabsboghandel Copenhagen 1965
7 Macapinlac M P Barney G H Pearson W N & Darby W J Production of zinc deficiency in the squirrel monkey (Saimiri sciureus) J Nutr 93 499 1967
8 Morrison S A Russell R M Carney E A & Oaks E V Zinc deficiency a cause of abnormal dark adaptation in cirrhotics Am J Clin Nutr 31 276 1978
9 Ott E A Smith W H Stob M & Beeson W M Zinc deficiency syndrome in the young lamb J Nutr 82 41 1964
10 Patek A J & Haig C The occurrence of abnormal dark adaptation and its relation to vitamin A metabolism in patients with cirrhosis of the liver J Clin Invest 18 609 1939
11 Plocke D J Levinthal C & Vallee B L Alkaline phosphatase of Escherichia coli a zinc metalloenzyme Biochemistry 1 373 1962
12 Simkin P A Oral zinc sulphate in rheumatoid arthritis Lancet 2 539 1976
13 Smith F R & Goodman W The effects of disease of the liver thyroid and kidneys on the transport of vitamin A in human plasma J Clin Invest 50 2426 1971
14 Smith J C McDaniel E G Fan F F & Halsted J A Zinc a trace element essential in vitamin A metabolism Science 181 954 1973
15 Smith J E Brown E D & Smith J C The effect of zinc deficiency on the metabolism of retinol binding protein in the rat J Lab Clin Med 84 692 1974
16 Sundaresan P R Cope F O & Smith J C Influence of zinc deficiency on retinal reductase and oxidase activities in rat liver and testes J Nutr 107 2189 1977
17 Vallee B L Wacker W E C Bartholomay A F & Robin E D Zinc metabolism in hepatic dysfunction I N Engl J Med 255 403 1956
18 Vallee B L Wacker W E C Bartholomay A F & Hoch F L Zinc metabol sm in hepatic dysfunction II N Engl J Med 257 1055 1957
19 Weismann K Acrodermatitis enteropathica treated with oral zinc sulphate Dan Med Bull 23 207 1976
20 — Intravenous zinc sulphate therapy in zinc depleted patients Dermatologica In press 1979
21 Weismann K Roed Petersen J Hjorth N & Kopp H Chronic zinc deficiency syndrome in a beer drinker with a Billroth II resection Int J Dermatol 15 757 1976
22 Weismann K Wadskov S Mikkelsen H I Knudsen L Christensen K Chr & Storgaard L Acquired zinc deficiency dermatosis in man Arch Dermatol 114 1509 1978
23 Weser U Seeber S & Warnecke P Zur Wirkung von Zn^{2+} auf die nukleare RNA und Proteinsynthese der Rattenleber Z Naturforsch B 24 866 1969

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Reversibility of Cardiovascular Changes in Extreme Obesity

Effects of Weight Reduction through Jejunoileostomy

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ABSTRACT The effects of marked weight reduction on cardiovascular function in extreme obesity were studied before and on average 2 years after jejunoileostomy In 17 female and 5 male patients mean age 36 years with obesity as the sole diagnosis circulatory data were obtained at rest and during exercise through right heart catheterization Blood volume and heart volume were measured and ECG was recorded at rest and during exercise A mean weight loss of 58 1 kg reduced percentage overweight from 104 to 39% Resting oxygen consumption and cardiac output fell in proportion to weight loss A slightly hyperkinetic central circulation was observed during exercise Stroke volume fell parallel to the decrease in blood volume and heart volume Systemic arterial pressure declined, while systemic arterial resistance did not change Left ventricular stroke work diminished Although lower than preoperatively, the filling pressures of the right and left side of the heart were high in relation to cardiac output compared with healthy subjects ECG at rest and during exercise remained normal Thus despite reductions in heart size and left ventricular stroke work evidence of left ventricular dysfunction persisted The observations indicate that the circulatory effects of gross obesity are largely reversible over weight loss periods of up to 3 5 years apart from signs of a reduced myocardial wall compliance The clinical implication seems to be that therapeutic weight reducing intervention should be made at a younger age

Key words blood volume cardiac output heart catheterization heart volume jejunoileostomy left ventricular stroke work obesity weight reduction

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Cardiovascular function in obesity has usually been evaluated by comparing the circulatory data of the obese patients with those of healthy subjects There have been very few proper analyses of the effects of obesity per se based on observations prior to and after weight loss The most extensive report (4) includes catheterization data of nine subjects who after caloric restriction for 4–36 months achieved a weight loss averaging 53 kg However as left ventricular filling pressures during exercise were recorded in only four subjects the observation of a persistent left ventricular dysfunction might not apply to gross obesity in general Due to the inclusion of a cardiovascular diagnosis important reservations must also be made about the finding of Sharma et al (22) concerning the effects of dietary restriction on symptoms ECG and central circulation in six moderately obese patients with angina pectoris Finally Slany et al (23) have described changes in the central circulation at rest observed af er 2–4 weeks of therapeutic starvation that lowered body weight from an average of 115 kg to 104 kg here too however there is the major drawback that six of the 12 subjects had additional diagnoses such as diabetes coronary heart disease and chronic alcoholism A later report (24) included circulatory data obtained during exercise but still from a similarly mixed patient group

Due to the potential hazards of starvation (13 16 25) and its brief effect on weight loss this zero diet regimen is being abandoned in favour of caloric restriction sometimes supported by psychological treatment But in cases of massive long standing

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Abbreviations P_{PA} = pulmonary artery pressure P_{PA_M} = mean P_{PA} P_{PCV_M} = mean pulmonary capillary venous pressure P_{BrA} = brachial artery pressure P_{BrA_M} = mean P_{BrA} P_{BrA_S} = systolic P_{BrA} P_{BrA_D} = diastolic P_{BrA} P_{RV} = right ventricular pressure P_{R} = systolic P_{R} $P_{R_{De}}$ = end-diastolic P_{RV}

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Table I *Physical characteristics of 22 patients (17 ♀ 5 ♂) preoperatively and after postoperative weight reduction (mean ± S E M)*

	Pre operative	Post operative	Significance[c]
Age (y)	34 7+1 9	36 6+1 9	***
Height (cm)	170 0+1 6	170 0±1 6	NS
Weight (kg)	146 0+4 0	87 9+2 9	***
Overweight[a] (%)	104 0+6 2	19 0±4 7	***
Broca s index[b]	2 1+0 06	1 3+0 04	***

[a] Excess weight in % of the ideal weight
[b] Weight (kg)/height (cm) − 100
Paired *t* tests NS not significant

overweight which defies other therapeutic efforts an alternative which has been considered in the past decade is an intestinal shunt operation In the present study a stable weight reduction was obtained by means of a small intestinal bypass operation Details concerning indications surgical techniques and management have been described elsewhere (18) In the investigation plan a threefold aim was defined 1) to provide further data on the cardiovascular adjustment to gross obesity 2) to make observations of the central circulation prior to and after weight reduction in order to analyze the effects of gross obesity per se and 3) hence to evaluate whether a cardiovascular indication for operative therapy should be added

SUBJECTS

Seventeen female and five male patients 17 51 years of age and weighing 115–176 kg were investigated immediately before jejunoileostomy They had been obese for several years and a dietary regimen or therapeutic starvation had achieved only temporary success In keeping with the selection criteria they had no other clinical diagnosis than obesity Coronary heart disease was excluded by a negative anamnesis and a normal ECG during and after exercise About two years after the operation their weight had stabilized after an average loss of 58 1 kg The second part of the study was then completed by repeating the investigative procedure The vital characteristics on the two occasions are presented in Table I

METHODS

Before heart catheterization the patients underwent a thorough investigation at the laboratory including exercise test with ECG recordings and determination of blood volume and heart volume

Ideal weight was calculated from height and a measure of skeletal frame the sum of the wrist widths (11 12) Overweight is expressed both as excess weight in per cent of the ideal weight and in terms of the commonly used Broca s index weight (kg)/(height (cm) − 100) ECGs were recorded with a direct writing ink jet recorder (Mingograf 61 and 81 Siemens Elema) both at rest and during and after exercise on an electrodynamically braked bicycle ergometer the work loads being increased stepwise every 6th min

Blood volume was measured with [131]I tagged albumin *Heart volume* was determined in the prone position (20)

Catheterization The right heart and the pulmonary artery were catheterized with a double lumen catheter (USCI size 8 F) introduced percutaneously into a cubital vein A short teflon catheter was introduced percutaneously into the brachial artery of the opposite arm

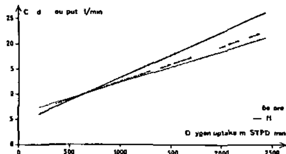
Pressures were measured with strain gauge transducers (Bell & Howell Ltd type 4-422) Zero pressure was measured at the midthoracic point of an anteroposterior plane at the insertion of the 4th rib

Heart rate was determined from ECG ECG and pre

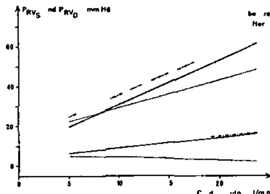
Table II *Circulatory data during right heart catheterization at rest and during supine exercise before (B) and after (A) weight reduction (mean + S D)*

Interposed are levels of significance between intraindividual observations NS not significant Number of observations given in parentheses

	Oxygen uptake (ml/min STPD)		A V difference (ml/l)		Cardiac output (l/min)		Heart rate (beats/min)	
	B	A	B	A	B	A	B	A
Rest	361 ±55 9 (22)	83 +40 3	45 0 NS +8 58 (22)	41 3 +6 08	8 5** +1 99 (21)	7 1 +1 53	78*** ±11 5 (2)	69 +10 4
Load I (16–98 W)	1 219 +315 1 (0)	1 061 +319 5	82 2 +10 95 (21)	75 9 +10 54	14 7 NS +3 14 (0)	13 8 +3 83	116 NS ±13 1 (22)	115 + 0
Load II (65–147 W)	1 662*** +355 6 (16)	1 453 +344 3	96 4 +12 57 (16)	87 2 +10 43	16 9* +2 44 (16)	16 0 +3 44	135 NS +19 6 (17)	136 + 0 8



F g 1 Regress on of card ac output at rest and durıng exe c se on oxygen consumpt on before and after we ght reduct on The th n unbroken l ne and the shaded a ea represent the regress on+2 S D ın healthy subjects (8 14 19)



F g 2 Relat on between P_{R_S} and $P_{R_{D_e}}$ and card ac out put at rest and durıng sup ne exerc se Reg ess on l nes as n F g 1

sures were recorded on an UV recorder (Osc llof1 S emens Stockholm Sweden) *Card ac oup t* was measured by the d rect Fick method Oxygen consump t on was determined by the Douglas bag techn que Blood samples were drawn simultaneously from the pulmonary and brach al arteries

The pulmonary res stance ndex was calculated as the d fference between the mean pressure n the pulmonary artery (P_{PA_M}) and the mean pulmonary cap llary venous pressure (P_{PCV_M}) divided by the card ac output and the system c res stance ndex as the mean p essure n the brach al artery (P_{BA_M}) d v ded by the card ac output Left ventricular stroke work was calculated as the product of stroke volume and (P_{BA_M} $P_{C M}$)

Current stat st cal methods have been appl ed Com parisons before and after we ght reduction were made w th the pa ed *t* test Reg ess on l nes were compared accord ng to Hald (17)

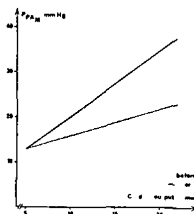
The catheterizat on commenced n the morn ng after a l ght meal All measurements were made at re t and durıng sup ne exerc se at two wo k loads These work loads were chosen ın the l ght of a p evıous exercise test ın the first nvest gat on In the second nvest gat on the same abso lute wo k loads were appl ed

RESULTS

Vital characterist cs of the 22 patients are given n Table I Tables II and III present the data obta ned durıng the measurements of card ac output at rest and durıng exercise at the same external loads be fore and after we ght reduct on The regress on of cardiac output on oxygen uptake and the relat on between intracardiac–intravascular pressures and cardiac output at rest and durıng exerc se before and after we ght reduction are dep cted n F gs 1 5

Before treat ne t As the data on the preoperat ve state before weight reduct on have been analyzed

Stroke volume (ml)	
B	A
109 +21 1 NS (21)	101 +20 0
1 8 -5 7 (0)	118 +24 2
1 7 18 1 (16)	117 +17 1



F g 3 P_{PA_M} n relat on to card ac output at re t and dur ıng exerc e Reg ess on l nes as n F g 1

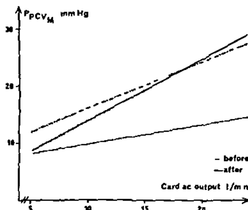


Fig 4 P_{PCV_M} in relation to cardiac ouput at rest and during exercise Regression lines as in Fig 1

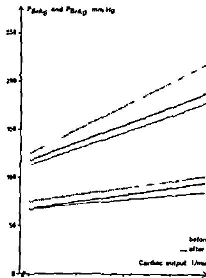


Fig 5 P_{BrA_S} and P_{BrA_D} in relation to cardiac output at rest and during exercise Regression lines as in Fig 1

separately (6 15) in 15 of the patients only a brief account will be given here The addition of 7 patients did not significantly alter the preoperative data The regression of cardiac output on oxygen uptake at rest and during exercise in the obese group was similar to that of healthy subjects indicating a normokinetic circulation The intracardiac and vascular pressures however attained higher values during exercise in the obese patients than in healthy controls Thus right ventricular (P_{RV}) and pulmonary arterial (P_{PA}) pressures displayed a steeper slope ($p<0\ 001$) on cardiac output while a higher intercept ($p<0\ 001$) was noted on pressure axes of the relations between P_{PCV_M} and all brachial arterial pressures and cardiac output respectively Among the male obese patients the stroke volumes and filling pressures of the left and right side of the heart were similar to those of healthy male athletes (7) The preoperative state of the central hemodynamics could be characterized as an adequate accomodation of circulatory dimensions and cardiac output to the metabolic demands but the pressure/flow relations within both the systemic and the pulmonary circulation indicated an increased load on the central circulation

Effects of weight reduction

About 23 months (range 10–38) after surgery body weight had stabilized after an average loss of 58 1 kg ($p<0\ 001$) corresponding to 65% of the calculated ideal weight Mean blood volume had de

Table III *Intracardiac and intravascular pressures (mmHg) during right heart catheterization at rest and during supine exercise before (B) and after (A) weight reduction (mean ± S D)*

Interposed are levels of significance between intraindividual observations Number of observations given in parentheses

	P_{RV_S}		$P_{RV_{De}}$		P_{PA_M}		P_{PCV_M}	
	B	A	B	A	B	A	B	A
Rest	30 ±10 (21)	24 ±5 1	9 ±4 7 (21)	8 ±3 6	20 ±7 3 (22)	16 ±3 3	12 ±4 5 (22)	11 ±3 5
Load I (16–98 W)	48*** ±14 9 (7)	40 ±6 5	17** ±12 7 (6)	11 ±2 4	33 ±9 7 (19)	27 ±8 8	22 ±7 9 (19)	17 ±5 9
Load II (65–147 W)	53 ±12.5 (18)	46 ±10 9	13 ±6 6 (18)	11 ±7 1	33 ±10 3 (20)	28 ±10 0	22 ±7 6 (18)	20 ±7 1

creased from 6 7 to 5 7 l ($p<0.001$) the ratio blood volume/kg body weight being increased ($p<0.001$) from 46 to 63 ml/kg Heart volume had been reduced by 10% to 890 ml ($p<0.05$) Resting and exercise ECGs remained normal The circulatory data obtained at rest and during exercise are given in Tables II and III and in Figs 1 5

At rest oxygen uptake had decreased by 78 ml/min ($p<0.001$) corresponding to 1 35 ml/min/kg weight loss Cardiac output showed a proportional decrease which was mainly due to a lower resting heart rate ($p<0.001$) hence the circulation remained normokinetic

During exercise on the same work loads as preoperatively the oxygen intake values were significantly lower as were the arteriovenous oxygen differences The relation between cardiac output and oxygen uptake (Fig 1) had changed significantly the slope of the regression line being steeper ($p<0.01$) than preoperatively and indicating a hyperkinetic circulation At comparable oxygen consumption levels the heart rate was higher and the stroke volume lower The reduction of the stroke volume was proportional to the decrease in blood volume and heart volume At rest and during exercise and at comparable values of cardiac output P_{RAs} had been lowered by 5 mmHg ($p<0.05$) while P_{RAde} had not changed significantly (Fig ?) All pulmonary arterial pressures had been significantly depressed by about 6 mmHg but P_{PCVM} remained unaltered (Figs 3 and 4) The pulmonary vascular resistance had not changed significantly All brachial arterial pressures were significantly decreased (Fig 5) Together with the decrease in cardiac output systemic vascular resistance was unaltered In all significant changes in the pressure flow relationships there was a lowering of the intercept the slope of the regression line being uninfluenced Left ventricular stroke work was significantly reduced

Comparison between treated patients and healthy subjects

The mean weight of the patients still represented an excess weight of 39% which may at least help to explain the subnormal ratio between blood volume and body weight (?0)

The relation between cardiac output and oxygen uptake (Fig 1) differed from that of the control group ($p<0.01$) as indicated by the steeper regression line for the patients This hyperkinetic circulation was an effect of a higher heart rate At any given cardiac output all pressures in the right ventricle pulmonary artery and in PCV position were higher than among the healthy subjects resulting in steeper regression lines (Figs ?–4) The regression lines of the brachial arterial pressures on cardiac output all had similar slopes to the healthy material but were displaced parallelly upwards (Fig 5) The differences at about 1? 3 l/min (average exercise blood flow)—7 6 and 4 mmHg for systolic mean and diastolic pressures respectively—were all significant although of small amplitude

DISCUSSION

It appears from previous and present data that the increased metabolic load imposed by obesity is met by enlargement of cardiovascular dimensions such as blood volume and heart size (? 3 6 9 10 ?1) Cardiac output and stroke volume have been reported to exceed the predicted values for ideal weight (1 4) but have correlated with weight gain (4) and oxygen consumption (4 6) In our investigative series certain cardiovascular data of the obese male patients were similar to those of healthy male athletes (7) viz large stroke volumes and total blood volumes and high cardiac filling pressures in relation to cardiac output Increased circulatory dimensions could thus per se contribute to the circulatory changes which if they reflected physiological adaptive processes would lead to contrary but interrelated changes after weight reduction

As mentioned in the introduction the

$P_{PA\,M}$	
B	A
10 +1? ?)	93 +1? 3
1 0 15 3 ?1)	107 +16 3
1 7 16 0 0)	11? +16 8

ity of the circulatory characteristics associated with uncomplicated obesity is poorly documented. Actually no reports on the hemodynamic effects of weight reduction through jejunoileostomy have been found in the literature. The only series with a comparable composition seems to be the nine patients investigated by Alexander and Peterson (4) before and after caloric restriction. The clinical diagnosis in their patients was exogenous obesity, endocrine disorders being excluded. Their report does not specify whether other concurrent diseases, e.g. ischemic heart disease or alcoholism, led to exclusion. The mean age, 38 years, was somewhat higher than in our patient group and the interval between the investigations was usually about half a year, in only two cases more than two years. The degree of overweight and the kilogrammes lost appear to be comparable, which also means that a relative overweight persisted at the second investigation. Similar effects of weight loss were observed in both studies, such as reductions of oxygen consumption, heart size and total blood volume. The decrease in resting cardiac output and systemic arterial pressure was associated with an unchanged systemic arterial resistance, but a diminished left ventricular stroke work. Exercise data from Alexander and Peterson's study are difficult to compare with the present series, as the intracardiac-intravascular pressures have not been related to cardiac output and represent only a few observations (mostly 4–6). The persistence of elevated cardiac filling pressures during exercise is, however, consistent with the present findings. Thus both studies have indicated that the weight loss reduced circulatory dimensions and diminished cardiac volume and pressure work load. The signs of continuing left ventricular dysfunction are substantiated by the present study. This change in myocardial function was not revealed by ECG, which demonstrated an ordinary pattern both at rest and during exercise. Gross and microscopic examinations of the myocardium in patients with marked and long standing obesity but without other chronic diseases with a conceivable cardiac effect (2, 5) have demonstrated an increase in heart weight and ventricular wall thickness together with a diffuse muscular hypertrophy. These necropsy findings might represent a morphological basis for a decreased myocardial compliance.

The hyperkinetic circulation observed after weight loss in the present study indicates a disturbed peripheral circulatory adaptation. As there was no anemia, the maladjustment of cardiac output is similar to that which may be seen in untrained individuals. The observation may imply that a training effect was lost with the reduction of excess weight. An alternative explanation would be that there had been no restriction of the fraction of cardiac output which was previously directed to the fatty tissues.

In conclusion, the present investigation shows that in extreme obesity a marked weight loss is associated with a reduction of previously enlarged circulatory dimensions, a lowering of systemic arterial pressure and a reduction of left ventricular stroke work. In contrast to these favourable effects there were, however, still signs of left ventricular dysfunction in these fairly young patients, which suggests that a cardiovascular indication for operative therapy of gross obesity should be added and that the operation should be performed in an early stage of a chronic obesity.

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REFERENCES

1. Alexander, J. K.: Obesity and cardiac performance. Am J Cardiol 14: 860, 1964.
2. Alexander, J. K., Amad, K. H. & Cole, V. W.: Observations on some clinical features of extreme obesity with particular reference to cardiorespiratory effects. Am J Med 32: 512, 1962.
3. Alexander, J. K., Dennis, E. W., Smith, W. G., Amad, K. H., Duncan, W. C. & Austin, R. C.: Blood volume, cardiac output, and distribution of systemic blood flow in extreme obesity. Cardiovasc Res Cent Bull Baylor Univ Coll Med 1: 39, 1962–63.
4. Alexander, J. K. & Peterson, K. L.: Cardiovascular effects of weight reduction. Circulation 45: 310, 1972.
5. Amad, K. H., Brennan, J. C. & Alexander, J. K.: The cardiac pathology of chronic exogenous obesity. Circulation 32: 740, 1965.
6. Backman, L., Freyschuss, U., Hallberg, D. & Melcher, A.: Cardiovascular function in extreme obesity. Acta Med Scand 193: 437, 1973.
7. Bevegård, S., Holmgren, A. & Jonsson, B.: Circulatory studies in well trained athletes at rest and during heavy exercise with special reference to stroke volume and the influence of body position. Acta Physiol Scand 57: 26, 1963.
8. — Effect of body position on the circulation at rest and during exercise, with special reference to the

influence on the stroke volume Acta Phys ol Scand 49 279 1960
9 Čermak J Das Herzvolumen bei Fettle b gen Das Herzvolumen und se ne Beziehung zur Korpergrosse zum Gewicht und zur Korpermasse bei obesen und proport onal entw ckel en Knaben Arch Kre slauf forsch 47 34 1965
10 Čermak J & Bosak V Das Herzvolumen bei Fett le b gen III M tt E nfluss des markanten Uber gew chtes auf das Herzvolumen be schwerarbe ten Mannern Arch Kre slaufforsch 62 12 1970
11 von Dobeln W An hropometric determinat on of fat free body we ght Acta Med Scand 165 37 1959
12 — Om begreppet o erv kt Lakartidn ngen 61 3988 1964
13 Dren ck E J Bladh W S nger G & Lederer M Body potass um content n obese subjects and potas s um deplet on during prolonged fast ng Am J Cl n Nutr 18 278 1966
14 Ekelund L-G & Holmgren A Central hemodynamics during exercise Circ Res (Suppl) 1 33 1967
15 Freyschuss U & Melcher A Exerc se energy ex pend tu e n extreme obes ty Influence of ergometry type and we ght loss Subm tted to Scand J Cl n Lab Invest 1977
16 Garnett E S Barna d D L Ford J Goodbody R A & Woodehouse M A Gross fragmentat on of card ac myof brils after therapeut c starvation for obes ty Lancet 1 914 1969
17 Hald A Statist cal theory w th engineering applica t ons pp 571 579 Wiley New York 1960
18 Hallberg D Backman L & Espmark S Surgical treatment of obes ty Prog Surg 14 46 1975
19 Holmgren A Jonsson B & Sjostrand T C rcula tory data in normal subjects at rest and during exercise n the recumbent pos t on with special reference to the stroke volume at different work ntens t es Acta Phys ol Scand 49 343 1960
20 Kjellberg S R Rudhe U & Sjostrand T The relat on of the cardiac volume to the we ght and surface area of the body the blood volume and the phys cal capacity for work Acta Radiol 31 113 1949
21 Schwalb H & Sch mert G Das Herz bei Fettsucht Med Kl n 65 1908 19 0
22 Sharma B Thadani U & Taylor S H Car d ovascular effects of we ght reduct on n obese patients with angina pectoris Br Heart J 36 854 1974
23 Slany J Mosslacher H Bodner P Irs gler K Lageder P Schmidt P & Schl ck W Card ovascu lar changes during therapeut c starvat on in obese subjects Kardio askulare Folgen e ner Nullkalonen d at bei Ad posen W en Kl n Wochenschr 86 443 1974
24 Slany J Mosslacher H & Irs gler K Bee nflusst Ad positas d e Herzfunktion? Z Kard ol 64 851 1975
25 Spencer I O B Death during therapeut c starvat on for obes ty Lancet 1 1 88 1968

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Abnormal Gamma Globulin Binding of Thyroid Hormones

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ABSTRACT Thyroid hormone levels were studied in a thyrotoxic patient, who was treated with propyl thiouracil He had heavily increased triiodothyronine concentrations measured by radioimmunoassay, in spite of only mild clinical symptoms of thyrotoxicosis A moderately increased serum triiodothyronine con centration was observed in another patient, who was euthyroid and who had recently recovered from subacute thyroiditis By gel electrophoresis and pre cipitation tests with human anti IgG and anti IgA a binding to the gamma globulins of both triiodothyronine and thyroxine was detected in patient 1, and of triiodothyronine in patient 2 Such abnormal binding may result in serious errors in the determina tion of thyroid hormone concentration by radioimmunoassay

Key words thyroid hormone antibodies thyroxine and triiodothyronine radioimmunoassay subacute thyroiditis

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About 80% of the serum thyroxine content is bound to the thyroxine binding globulin (TBG) which is an inter α globulin and 20% to albumin (12 15) and to the thyroxine binding prealbumin (2 9) In addition a binding of triiodothyronine to α 1 and β lipoproteins has been detected in normal human serum (7) by means of a sensitive radioimmunoelectrophoretic technique In recent years several cases have been reported in which the patient s clinical thyroid state has been inconsistent with the observed thyroid hormone levels in the serum as determined by radioimmunoassay An abnormal binding of thyroxine or triiodothyronine to the gamma globulins has been observed Most of the patients have been euthyroid A case of hypothyroidism possibly with antibodies against thyroxine and triiodothyronine as a pathogenetic factor how ever has been reported (8) We observed such an abnormal binding of thyroid hormones in two pa tients one having propylthiouracil treated thyrotoxicosis and the other subacute thyroiditis

CASE HISTORIES AND LABORATORY DATA

Case 1

Clinical thyrotoxicosis with diffuse goiter but without eye signs was diagnosed in a man aged 24 Thyroid scan had not been performed The competitive protein binding procedure of Murphy (10) revealed a serum thyroxine (T_4) concentration of 370 nmol/l (normal range 66–139) in Aug 1972 The patient was treated with methylthiouracil for a total period of about one year Thereafter he was euthyroid and his serum T_4 value was normal

The patient had a relapse in Aug 1975 and the serum T_4 concentration was 195 nmol/l He was treated with propylthiouracil for 1 year During the whole treatment period the serum T_4 was measured repeatedly fluctuating between 52 and 125 nmol/l

Serum triiodothyronine (T_3) values were determined in a commercial laboratory using a radioimmunological double antibody method modified according to Chopra et al (1) (normal range 1 14–2 30 nmol/l) From May 1976 heavily increased serum T_3 values were found (Fig 1) but in the same period the patient was clinically only mildly thyrotoxic indicating that the T_3 assay was hampered by an analytic source of error presumably an abnormal gamma globulin binding of T_3 in the patient s serum

Serum T_4 was determined in Aug 1976 by radioimmunoassay and also by a competitive protein-binding procedure (10) Both methods showed normal serum T_4 levels Serum T_3 concentration was measured at the same time by radioimmunoassay Dextran-coated charcoal was used in our laboratory for separation of free and antibody bound hormone in the radioimmunoassay This resulted in a serum T_3 value of 10 2 nmol/l (normal range 1 6–2 8) whereas a value of 111 nmol/l was obtained by the double antibody procedure used in the commercial laboratory (normal range 2 14–2 30)

The true serum T_3 content was evaluated by performing a radioimmunological determination on an alcoholic extract of serum This procedure resulted in a value of 3 9 nmol/l (normal range 1 6–2 8) This increase above normal may probably be explained by the gamma globulin binding of T_3 and may also explain the result of the T_3-resin uptake test which was 0 41 (normal range 0 80–1 25)

Abbreviations TBG = thyroxine binding globulin TSH = thyroid stimulating hormone TRH = thyrotropin-releasing hormone T_3 = triiodothyronine T_4 = thyroxine

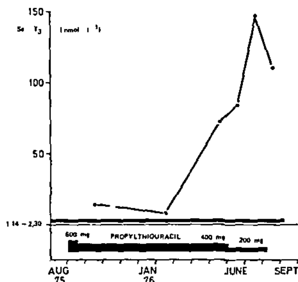


Fig 1 Serum T_3 values in case 1 determined by radioimmunoassay (double antibody procedure)

Serum TBG was normal (24 8 mg/l) Serum thyroid stimulating hormone (TSH) was less than 0 4 mU/l (normal <1 9) and there was no TSH response to the thyrotropin releasing hormone (TRH) stimulation In June 1976 the basal metabolic rate was +35% in two measurements confirming the clinical impression of a mild thyrotoxicosis A positive reaction to the thyroglobulin antibodies (CA 2 antibody immunofluorescence technique) was detected in serum as well as a moderate positive reaction to cytoplasmatic thyroid antibody Thyroid stimulating antibodies were not determined

Examinations for thyroid hormone binding antibodies

One μCi ^{125}I labelled T_4 (Abbott ® specific activity 1 172 μCi/μg corresponding to a serum concentration of 2 2 nmol/l) was added to 0 5 ml of the patient s serum One μCi ^{125}I labelled T_3 (specific activity 465 μCi/μg corresponding to a serum concentration of 6 6 nmol/l) was added to another 0 5 ml serum sample and both were incubated at 37°C for 30 min after which an agar gel electrophoresis in diemal buffer pH 8 6 and ionic strength 0 075 was performed Duplicate tests of 15 μl serum were applied to the gel and the electrophoresis was performed at a potential difference of 12 V/cm at 20°C for 55 min Subsequently one of the gels was stained in order to identify the individual serum protein bands and the other was cut up into strips of 5 mm which were counted separately in a gamma scintillation counter By comparing these two results the binding of the labelled thyroid hormones to the serum protein fractions could be determined Normal serum was controlled by similar tests

In other experiments 5 μl of the patient s serum were *labelled with ^{125}I T_3 mixed with 300 μl human anti IgG* anti IgA and 1500 μl polyethylenglycol incubated at 20 C for 20 min and centrifuged for 10 min The radioactivities of both the supernatant and the precipitate were measured and the ratio between them was calculated and compared with an equivalent ratio obtained by identical tests with normal human serum containing ^{125}I T_3

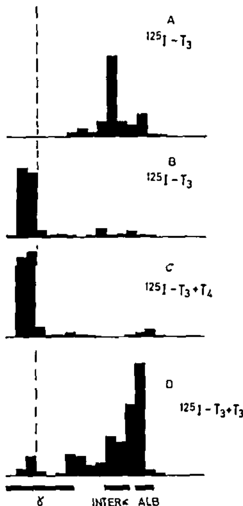


Fig 2 Gel electrophoresis of ^{125}I T_3 labelled serum from a normal patient (*A*) and from case 1 (*B D*) demonstrating abnormal gamma globulin binding of T_3

Results of examination for thyroid hormone binding antibodies

Electrophoresis of serum with added ^{125}I T_3 revealed an abnormal binding of T_3 in the gamma globulin area and only a weak binding to the inter-α globulins (corresponding to TBG) prealbumin and albumin (Fig 2*B*) The addition of non radioactive T_4—to a concentration of 2 6×10^5 nmol/l—in the ^{125}I T_3 labelled serum intensified the accumulation in the gamma globulin area (Fig 2*C*) whereas the addition of non labelled T_3—to a concentration of 1 5×10^3 nmol/l—displaced the major part of the ^{125}I T_3 from the gamma globulin area (Fig 2*D*) In similar tests with ^{125}I T_4 added to the serum an abnormal binding of T_4 in the gamma globulin area was detected though to a smaller extent than for T_3

In the experiments with addition of human anti IgG and *anti IgA to the patient s* [125]I T_3 *labelled serum the ratio* between the radioactivity in the precipitate and in the supernatant was 0 414 A corresponding determination for normal serum gave a ratio of 0 033 These results confirm the abnormal binding of T_3 to the gamma globulins

Case 2

In a clinically euthyroid woman aged 47 who had just recovered from typical subacute thyroiditis elevated serum T_3 concentration 3 8 nmol/l was observed 8 months after the onset of the disease At the same time her serum T_4 concentration was 78 7 nmol/l and serum *TSH concentration 2 5 mU/l Binding of* T_3 *but not of* T_4 to the gamma globulins was found although not so pronounced as in case 1 Thyroid autoantibodies had not been determined

DISCUSSION

In the serum of patient 1 we detected an abnormal binding of T_4 and especially of T_3 to the gamma globulins The affinity of T_3 to the binding sites in the gamma globulin area seemed to be higher than to the thyroid hormone binding proteins in the inter α area to prealbumin and to albumin Addition of large quantities non labelled T_3 displaced the [125]I T_3 from the gamma globulin area showing that the gamma globulin binding sites were specific for T_3 A similar specificity was observed for the T_4 binding to the gamma globulins

In the serum of patient 2 binding sites for [125]I T_3 were found in the gamma globulin area but no T_4 binding antibodies could be detected In contradistinction to patient 1 serum T_3 was only slightly elevated and the increase would not in itself arouse suspicion of abnormal gamma globulin binding This illustrates the necessity of using a radio immunological procedure which involves the determination of such binding phenomena in each serum tested

Binding of T_4 to gamma globulins was reported for the first time in 1956 by Robbins et al (16) in a woman who had undergone two operations for a papillary thyroid carcinoma The first attempt to explain the appearance of such abnormal binding to gamma globulins was made in 1963 by Premachandra et al (14) By immunization of guinea pigs with thyroglobulin in Freund s adjuvant they were *able to demonstrate the formation of antibodies* against T_3 and T_4 and therefore in their opinion these hormones seemed to form a kind of association with an antithyroglobulin complex In 1972 Ochi et al (11) discovered that if serum with a high antibody titer against T_4 was absorbed with thyroglobulin the antibody titer against T_4 as well as thyroglobulin decreased These authors also immunized rabbits with denatured thyroglobulin and found an abnormal binding of T_3 and T_4 but not of diiodothyronine or *l* tyrosine A denaturation process will open the polypeptide chains of the thyroglobulin molecule and it may be speculated that T_3 and T_4 are exposed in this manner to the antigenic apparatus and are acting as haptens Herrmann et al (6) came to a similar conclusion after examination of serum from a girl with Hashimoto s thyroiditis The cause of such a possible denaturation in vivo is not apparent The presence of thyroglobulin antibodies however is not a prerequisite for gamma globulin binding of T_3 and T_4 according to observations by Staehelı et al (17) and Wu and Green (20) who have also discussed hereditary protein abnormalities or monoclonal gammopathy as possible causes

Antibodies against T_4 have been reported in several patients suffering from Hashimoto s thyroiditis (3 5 11 13 17 20) most of them with a high antithyroglobulin titer In addition T_3 and/or T_4 antibodies have been reported in patients suffering from thyroid cancer (16) primary hypothyroidism hyperthyroidism and in one patient with secondary hypothyroidism treated with desiccated thyroid (17)

The increasing interest in high serum gamma globulin binding of thyroid hormones is due to the fact that this phenomenon may cause serious errors in the radioimmunoassay of these hormones As reported by Wu and Green (20) and Herrmann et al (5) abnormal thyroid hormone binding gamma globulins may result in either false high or false low serum concentrations of T_3 and T_4 as measured by radioimmunoassay If the antibody bound T_3 is separated from the free T_3 by charcoal (5) the increased quantity of radioactivity in the antibody bound fraction will result in a too low serum T_3 concentration *If* on the other hand a double antibody method is used for the separation of free and antibody bound T_3 as for instance in the method of Chopra et al (1) a decreased radioactivity in the double antibody bound fraction will result in a *falsely high serum* T_3 *value The alcoholic extrac*tion procedure gave a true serum T_3 value of 3 9 nmol/l

However using the charcoal separation procedure we found the high serum T_3 value of 10 2

nmol/l (normal range 1 6–2 8) Our calculations of the latter value include a correction for the gamma globulin binding but when values for such binding are extremely high this correction does not give reliable values Therefore it is important to realize that the radioimmunoassay procedure for T_3 and T_4 determinations is not reliable when serum gamma globulin binding phenomena are observed and that correction procedures often will be inadequate In such cases a determination of serum T_3 in an alcoholic extract of serum or a determination of the free non protein bound T_3 by a dialysate procedure (18) should be preferred

Almost all the patients with serum gamma globulin binding of the thyroid hormones have been clinically euthyroid in spite of a high binding of these hormones to the gamma globulins This finding must indicate that the free active hormone fraction is more or less unaltered In previous work by Premachandra and Blumenthal (13) however the possibility has been discussed that a strong gamma globulin binding of T_3 might cause hypothyroidism owing to a too low free hormone concentration In 1977 Hehrmann et al (4) reported on a patient who was euthyroid with normal serum T_3 and T_4 values but with severely increased TSH response to TRH stimulation Recently Karlsson et (8) reported on a woman aged 26 who in the urse of one year developed a clinical myxoedema high TSH and low serum T_4 but with strongly increased serum T_3 concentration measured by a double antibody radioimmunoassay procedure (19) Fine needle biopsies showed normal thyroid cells with no increase in the number of lymphoid cells A very strong gamma globulin binding of T_3 was detected while the binding of T_4 only was poor It seems reasonable to assume that this patient had formed such a considerable amount of T_3 antibodies that the formation of T_3 had not been able to keep pace with it resulting in a decrease in the free T_3 fraction Therefore it cannot be excluded that a strong gamma globulin binding may result in a transient hypothyroid state

The use of radioimmunoassay for the determination of *antibodies* against T_3 and T_4 (4) has made it possible to evaluate the frequency of thyroid hormone binding to the gamma globulins The result was that such antibodies could be detected in 5 out of 3000 sera (4) Consequently gamma globulin binding of thyroid hormones does not occur very often

Such binding phenomena should be suspected however in all cases where the results of radioimmunological determination of T_3 and of T_4 disagree with the clinical impression It should be emphasized that serious mistakes may be avoided if the radioimmunoassay procedure includes an evaluation of binding of T_3 and T_4 to the gamma globulins There is no satisfactory explanation for the production of these autoantibodies against the thyroid hormones in cases like the present ones This problem awaits further experimental elucidation

REFERENCES

1 Chopra J C Ho R S & Lam R J J Lab Clin Med 80 729 1972
2 Davis P J Handwerger S & Gregerman R I J Clin Invest 5 515 1972
3 Ginsberg J Segal R N Ehrlich R M & Walfish P G Clin Endocrinol 8 133 1978
4 Hehrmann R Hoffken B von zur Muhlen H Greutzig H Thiele J & Hesch R D Horm Metab Res 9 326 1977
5 Herrmann J Kley K H Kroll H J & Kruskemper H L Dtsch Med Wochenschr 101 966 1976
6 Herrmann J Rudorff K H Kroner H & Premachandra B N Horm Metab Res 9 394 1977
7 Itoh K F Miyai K Kimura K Abe H & Kumahara Y Clin Chim Acta 30 259 1970
8 Karlsson F A Wibell L & Wide L N Engl J Med 296 1146 1977
9 Larsen P R J Clin Invest 51 1125 1972
10 Murphy J J Clin Med 66 161 1965
11 Ochi Y Shiomi K Hashiya T Yoshimura M & Miyazaki T J Clin Endocrinol Metab 35 743 1972
12 Oppenheimer J H & Surks M I In Handbook of physiology vol III sect 7 (ed M A Greer and D H Solomon) p 167 American Physiological Society Washington D C 1974
13 Premachandra B N & Blumenthal H T J Clin Endocrinol Metab 27 931 1967
14 Premachandra B N Ray A K Hirata Y & Blumenthal H T Endocrinology 73 135 1963
15 Robbins J & Rall J E Physiol Rev 40 415 1960
16 Robbins J Rall J E & Rawson R W J Clin Endocrinol Metab 16 573 1956
17 Staeheli V Vallotton M B & Burger A J Clin Endocrinol Metab 41 669 1975
18 Weeke J & Orskov H Scand J Clin Lab Invest 35 237 1975
19 Wide L Acta Endocrinol (Kbh) (Suppl) 142 207 1969
20 Wu S Y & Green W L J Clin Endocrinol Metab 42 642 1976

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Corticosteroids and Thyroid Function

Different Effects on Plasma Volume Thyroid Hormones and Thyroid Hormone Binding Proteins after Oral and Intravenous Administration

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ABSTRACT The influence of glucocorticosteroids on plasma volume, thyroid hormones and thyroid hormone binding proteins was studied in 17 patients Plasma volume was not affected either by i v beta methasone (6 mg daily) or by oral prednisolone (45–180 mg daily) given for 5 days The serum T_3 concentration decreased while rT_3 increased in dependently of the route of administration of corticosteroids Serum T_4 concentration decreased after i v but not after oral administration of corticosteroids Oral steroids as compared to i v in creased the ^{125}I triiodothyronine uptake test value The serum TBG concentration decreased independ ently of the route of administration, while the se rum TBPA concentration increased after oral cor ticosteroids but was unchanged after i v treat ment The serum TSH concentration was slightly re duced About half of the patients were given both corticosteroids and nutrition i v and the other half were given all treatment by mouth The part played by the route of administration of corticosteroids and calories, respectively, cannot be evaluated at present but these factors seem to be of importance

Key words glucocorticosteroids plasma volume thyroid hormones thyroid hormone binding proteins

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The thyroid uptake of iodine is decreased and its urinary excretion increased after administration of corticosteroids (12 14 17 31) Corticosteroids also induce a decrease in the concentration of protein bound iodine (PBI) in blood (2 12) and this effect is partly caused by a decrease in the serum concentra tion of thyroxine binding globulin (TBG) (22) and partly by an increase in plasma volume (2) The serum triiodothyronine (T_3) concentration is re duced after corticosteroid administration (5 8 9 10 15 23) while that of the metabolically inactive compound reverse triiodothyronine (rT_3) is in creased (3 6 28) presumably due to an altered peripheral metabolism of thyroxine (T_4)

The aim of the present investigation was to study whether change in plasma volume could contribute to the observed alteration in the T_3 level after corticosteroid administration Unexpectedly oral and i v corticosteroid administration had different effects on the thyroid tests These results will also be reported

PATIENTS AND METHODS

The 17 patients studied suffered from either chronic in flammatory bowel disease or hematological illness and the corticosteroids were part of their treatment The age range was 24–76 years (mean 49)

Nine patients with ulcerative colitis or Crohn s disease were treated with i v betamethasone (Betapred®) 3 mg twice daily They were kept on total parenteral nutrition except for small amounts of water orally Eight patients with various blood diseases were treated with pred nisolone orally 45–180 mg (mean 86) per day in divided doses all of these patients were fed by mouth and did not receive any drug with known effect on thyroid function

Before therapy with corticosteroids was started blood samples were drawn after an overnight fast and serum was separated for chemical analyses The plasma volume was determined with ^{125}I albumin according to Fine and Seligman (11) After 5 days on corticosteroid therapy the same procedure was repeated

The concentration of T_4 in serum was determined by a competitive protein binding technique (24) using a purified fraction of TBG instead of serum from pregnant women used in the original method

Abbreviations PBI=protein bound iodine TBG=thyrox ine binding globulin T_3=triiodothyronine rT_3=reverse T_3 T_4=thyroxine TSH=thyroid stimulating hormone TBPA=thyroxine binding prealbumin

Table I *Thyroid function before (B) and after (A) corticosteroid treatment*

Pat no	Corticosteroid dosage (mg)	Sex	Age (y)	Plasma volume (l) B	Plasma volume (l) A	T_4 (nmol/l) B	T_4 (nmol/l) A	T_3 (nmol/l) B	T_3 (nmol/l) A	rT_3 (nmol/l) B	rT_3 (nmol/l) A	^{125}I T_3 uptake test (%) B	^{125}I T_3 uptake test (%) A
Betamethasone i v													
1	6	♂	26	2 5	2 5	66	68	2 2	1 5	0 236	0 277	91	99
2	6	♂	25	2 9	2 9	118	93	2 3	2 0	0 480	0 501	68	64
3	6	♂	56	2 3	2 9	130	120	1 6	2 0	0 464	0 516	83	89
4	6	♂	36	3 1	2 9	89	78	2 4	1 8	0 447	0 510	119	121
5	6	♀	62	2 4	2 3	97	80	1 0	0 9	0 536	0 711	124	160
6	6	♀	60	2 9	2 3	138	126	3 4	1 7	0 394	0 602	79	92
7	6	♀	36	2 6	2 7	128	100	2 4	1 6	0 537	0 747	81	76
8	6	♂	46	3 3	3 3	121	86	2 4	1 5	0 476	0 510	60	98
9	6	♂	27	3 5	4 3	111	106	2 0	1 4	0 434	0 462	68	110
Mean			42	2 8	2 9	111	95	2 2	1 6	0 445	0 537	90	101
S D				0 4	0 6	23	20	0 7	0 3	0 091	0 139	19	28
t				0 32		4 24		−3 24		3 45		1 59	
p				N S		<0 01		<0 02		<0 01		N S	
Prednisolone orally													
10	60	♂	76	3 2	3 5	84	61	2 0	1 2	0 294	0 470	96	99
11	100	♀	58	2 3	2 0	81	73	2 2	1 0	0 271	0 472	95	108
12	60	♀	27	2 3	2 3	83	52	1 1	0 5	0 206	0 340	98	116
13	45	♂	64	3 2	3 0	91	62	1 9	1 5	0 260	0 375	88	98
14	180	♀	70	2 2	3 4	116	143	2 2	1 7	0 284	0 893	73	106
15	60	♀	24	2 3	2 6	118	98	2 0	1 7	0 366	0 597	105	115
16	45	♀	67	–	–	94	114	1 6	1 2	0 693	0 986	99	118
17	60	♀	73	3 4	3 4	116	131	1 8	1 6	0 582	0 693	90	114
Mean			57	2 7	2 9	98	92	1 9	1 3	0 370	0 603	93	109
S D				0 5	0 6	16	35	0 4	0 4	0 163	0 222	10	8
t				0 98		−0 74		−4 88		4 04		5 73	
p				N S		N S		<0 01		<0 01		<0 001	
ll patients													
			49	2 8	2 9	105	94	2 0	1 5	0 409	0 568	92	105
S D				0 5	0 6	21	27	0 6	0 4	0 132	0 188	15	21
t						−2 57		−5 22		4 61		3 45	
p				N S		<0 05		<0 001		<0 001		<0 01	
Reference value						60–140		1 2–2 5		0 14–0 54			

The T_3 concentration in serum was measured by radioimmunoassay (13) with a double antibody technique using mertiolate as the displacer of T_3 from thyroid hormone binding proteins

The ^{125}I triiodothyronine uptake test was performed using Sephadex as the adsorbent (20)

The thyroid stimulating hormone (TSH) in serum was measured by radioimmunoassay (21) with MRC human pituitary TSH 68/38 as a reference standard

TBG was measured by radial immunodiffusion (16)

The thyroxine binding prealbumin (TBPA) was measured by electroimmunoassay (18) Stabilized standard human serum from Behringwerke AG was used as a standard

rT_3 was measured by radioimmunoassay (4) using polyethylene glycol precipitation The kit was from Biodata Italy

RESULTS

The detailed results are shown in Table I Neither betamethasone nor prednisolone changed the plasma volume

The serum concentration of T_3 was significantly reduced by corticosteroids (p<0 001) while that of rT_3 was increased (p<0 001) The serum T_3 was not affected by the route of administration of the steroids (p=0 17 N S) but rT_3 increased more after oral than after i v therapy (t=2 30 p<0 02)

The serum T_4 concentration was reduced after corticosteroids when the total group was considered (p<0 05) but this was caused by a marked reduction after i v administration (p<0 01) while

TSH (μU/ml)		TBG (mg/l)		TBPA (%)	
B	A	B	A	B	A
3 4	5 2	17 0	13 4	0 196	0 228
[illegible] 4	1 0	17 2	13 9	0 258	0 228
1 9	0 9	16 1	16 6	0 200	0 272
6 1	2 4	9 7	9 1	0 340	0 240
2 4	0 8	13 7	8 0	0 050	0 120
0 5	0 9	22 6	18 3	0 190	0 156
1 1	<0 5	20 8	16 5	0 224	0 228
1 1	<0 5	19 9	14 2	0 228	0 240
0 7	<0.5	14 4	14 3	0 200	0 194
2 [illegible]	1 4	16 8	13 8	0 210	0 212
1 8	1 5	4 0	3 4	0 076	0 047
−1 53		−3 81		1 52	
N S		<0 01		N S	
1 4	<0 5	17 4	12 8	0 236	0 276
1 4	<0 6	17 3	14 1	0 272	0 319
1 2	0 8	16 3	12 5	0 165	0 311
2 7	2 9	18 0	14 3	0 145	0 279
2 1	0 9	24 4	16 6	0 176	0 287
3 1	3.5	14 1	11 9	0 248	0 290
3 2	0 8	15 1	13 7	0 693	0 986
1 2	0 6	18 5	15 3	0 431	0 582
2 0	1 3	17 6	13 9	0 212	0 297
0 9	1 2	3 1	1 5	0 045	0 021
−2 31		−5 53		−5 41	
N S		<0 001		<0 001	
[illegible] 1	1 4	17 2	13 9	0 211	0 252
1 4	1 3	3 5	2 6	0 061	0 57
−2 53		−6 48		2 56	
<0 05		<0 001		<0 05	
<7 0		♂ 9 8–17 8 ♀ 11 3–20 5		80–120	

the levels were not significantly affected by steroids given by mouth

The serum TBG concentration was reduced irrespective of the mode of administration of corticosteroids The change seemed to be more pronounced after the oral ($p<0\ 001$) than after the i v ($p<0\ 01$) route but the difference between the two groups did not achieve statistical significance ($t=0\ 658$ N S)

The correlation between the serum concentrations of T_3 and TBG was not significant before ($r=0\ 447$ N S) or after treatment ($r=0\ 394$ N S) but a significant correlation was achieved when the results for all patients both before and after corticosteroid administration were calculated ($r=0\ 575$ $p<0\ 001$)

^{125}I triiodothyronine uptake test value increased during corticosteroid therapy when the total group was considered ($p<0\ 01$) This was due to a marked increase after the oral administration ($p<0\ 001$) i v administration did not affect the result

The serum TBPA concentration increased during corticosteroid therapy ($p<0\ 05$) This was related to a marked increase after corticosteroids given by mouth ($p<0\ 001$) while i v administration of corticosteroids had no such effect

There was a moderate fall of the serum concentration of TSH after corticosteroids ($p<0\ 05$)

DISCUSSION

Corticosteroids promote a reduction of the serum T_3 concentration (5 8 9 10 15 23) This is presumably due to a change in the metabolism of T_4 so that after its deiodination more rT_3 and less T_3 are produced However it has also been suggested that the lowered T_3 may be partly due to a change in the distribution volume of the hormone (27) Blomstedt et al (2) found that the reduction in serum PBI after administration of hydrocortisone (100 mg × 3 for four days) was partly brought about by an increase in plasma volume We were unable to confirm this finding and we believe that the alteration of thyroid hormone concentration is not secondary to an increase in plasma volume This discrepancy of results may be due to the more pronounced mineralocorticoid effect of hydrocortisone compared with betamethasone and prednisolone (26) Indeed Westgren et al (28) have shown that mineralocorticoids in contrast to glucocorticoids do not influence the serum levels of T_4 T_3 and rT_3 The total distribution volume of the thyroid hormones has not been studied and it is still unknown whether corticosteroids induced an increase in the intracellular T_3 concentration with an extracellular decrease as a result

T_3 is mainly transported by TBG although a small amount can be bound to TBPA (7) The relative importance of the serum TBG concentration for the serum level of T_3 is difficult to evaluate as a positive correlation was achieved only when a fairly large number of observations were made However such a connection seems to exist and therefore it cannot be ruled out that the decrease in serum TBG

could contribute to the reduced serum T_3 On the other hand this does not explain the increase in serum rT_3

Another cause of the fall in T_3 after corticosteroids could be the decreased secretion from the thyroid as suggested in an earlier study (25) This fall could possibly be mediated via suppression of TSH (30) or TRH secretion (25) Vigneri et al (27) found that TSH stimulation induced the same percentage increase in serum T_3 concentration after as well as before corticosteroids Thus steroids do not seem to suppress hormone secretion from the thyroid gland directly

It follows that the most likely explanation for the decrease in serum T_3 and the increase in serum rT_3 after corticosteroid administration is a diversion of the metabolic deiodination of T_4 as reported by other workers (3 6 28)

The route of administration of the corticosteroids seems to be of importance For instance oral corticosteroids induced a reduction of the TBG concentration while the T_4 concentration was unchanged resulting in an increase in the ^{125}I triiodothyronine uptake test value Corticosteroids i v reduced both the TBG and the T_4 concentrations with an unaltered ^{125}I triiodothyronine uptake test value The TBPA concentration increased considerably after oral but was unaffected after i v administration The T_3 concentration was reduced irrespective of the route of administration on the other hand rT_3 increased more after oral than after i v corticosteroid treatment The reason for these findings is unknown but it is tempting to speculate about the importance of the corticosteroid concentration in portal blood that should be higher in patients on oral than i v corticosteroids Since the deiodination of T_4 to T_3 occurs in the liver (1 19) such a factor could be of importance

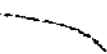
It is unlikely that the clinical condition of the patients altered during the five-day study so as to influence the results presented However it should be noted that some patients received both corticosteroids and their nutrition orally while others were treated i v in both respects The route of administration of calories may be important Westgren et al (29) have shown that alterations in T_3 and rT_3 concentrations induced by fasting are normalized after oral but not i v glucose administration As far as we know no study has been made of whether such factors also influence the concentration of the thyroid hormone binding proteins We are at present trying to evaluate their relative importance

REFERENCES

1 Becker D V & Prudden J F The metabolism of I^{131} labeled thyroxine triiodothyronine and diiodotyrosine by an isolated perfused rabbit liver Endocrinology 64 136 1959
2 Blomstedt B & Einhorn J Effect of cortisone on the PBI and resin uptake of I^{131}-triiodothyronine Metabolism 16 319 1967
3 Burr W A Ramsden D B Griffiths R S Black E G Hoffenberg R Meinhold H & Wenzel K W Effect of a single dose of dexamethasone on serum concentrations of thyroid hormones Lancet 2 58 1976
4 Chopra I J A radioimmunoassay for measurement of 3 3 5 triiodo-thyronine (reverse T_3) J Clin Invest 54 583 1974
5 Chopra I J Chopra U & Orgiazzi J Abnormalities of hypothalamo-hypophyseal thyroid axis in patients with Graves ophtalmopathy J Clin Endocrinol Metab 37 955 1973
6 Chopra I J Williams D E Orgiazzi J & Solomon D H Opposite effects of dexamethasone on serum concentrations of 3,3 5 triiodothyronine (reverse T_3) and 3,3 ,5 triiodothyronine (T_3) J Clin Endocrinol Metab 41 911 1975
7 Davies P J Handwerger B S & Gregerman R I Thyroid hormone binding by human serum prealbumin (TBPA) J Clin Invest 51 515 1972
8 Degroot L J & Hoye K Dexamethasone suppression of serum T_3 and T_4 J Clin Endocrinol Metab 42 976 1976
9 Duick D S Warren D W Nicoloff J T Otis C L & Croxson M S Effect of single dose dexamethasone on the concentration of serum triiodothyronine in man J Clin Endocrinol Metab 39 1151 1974
10 Emerson C H & Utiger R D Hyperthyroidism and excessive thyrotropin secretion N Engl J Med 287 328 1972
11 Fine J & Seligman A M Traumatic shock study of problem of lost plasma" in hemorrhagic shock by use of radioactive plasma protein J Clin Invest 22 285 1943
12 Fredrickson D S Forsham P H & Thorn G W The effect of massive cortisone therapy on measurements of thyroid function J Clin Endocrinol Metab 12 541 1952
13 Gharib H Ryan R J & Mayberry W E Radioimmunoassay for triiodothyronine (T_3) I Affinity and specificity of the antibody for T_3 J Clin Endocrinol 33 509 1971
14 Hill Jr S R Reiss R S Forsham P H & Thorn G W The effect of adrenocorticotropin and cortisone on thyroid function Thyroid-adrenocortical interrelationships J Clin Endocrinol Metab 10 1375 1950
15 Jarnerot G The thyroid in ulcerative colitis and Crohn s disease Thesis Linkoping 1974

16 Kågedal B & Kallberg M Determination of thyroxine binding globulin in human serum by single radial immunodiffusion and radioimmunoassay Clin Chem 23 1694 1977
17 Kuhl Jr W J & Ziff M Alteration of thyroid function by ACTH and cortisone J Clin Endocrinol Metab 12 554 1952
18 Laurell C B Electroimmunoassay Scand J Clin Lab Invest (Suppl) 124 21 1972
19 Nomura S Pittman C S Chambers Jr J B Buck M W & Shimizu T Reduced peripheral conversion of thyroxine to triiodothyronine in patients with hepatic cirrhosis J Clin Invest 56 643 1975
20 Nosslin B A simplified technique for the triiodothyronine test (T_3 test) with Sephadex Scand J Clin Lab Invest (Suppl) 86 177 1965
21 Odell W D Rayford P L & Ross G T Simplified partially automated method for radioimmunoassay of human thyroid stimulating growth luteinizing and follicle stimulating hormones J Lab Clin Med 70 973 1967
22 Oppenheimer J H & Werner S C Effect of prednisone on thyroxine binding proteins J Clin Endocrinol 26 715 1966
23 Re R N Kourides I A Ridgway E C Weintraub B D & Maloof F Glucocorticoid effect on TSH and prolactin secretion Clin Res 22 347A 1974
24 Seligson H & Seligson D Measurement of thyroxine by competitive protein binding Clin Chim Acta 38 199 1972
25 Singer P A & Nicoloff J T Estimation of triiodothyronine secretion rate in euthyroid man J Clin Endocrinol Metab 35 82 1972
26 Sjogren B Hormoner och hormonterapi 191 Leo Goteborg 1973
27 Vigneri R Pazzino V Filetti S Squatrito S Galbiati A & Polonsa P Effect of dexamethasone on thyroid hormone response to TSH Metabolism 24 1209 1975
28 Westgren U Ahrén B Burger A Ingemansson S & Melander A Effects of dexamethasone desoxycorticosterone and ACTH on serum concentrations of thyroxine 3 5,3 triiodothyronine and 3 5 3 triiodothyronine Acta Med Scand 202 89 1977
29 Westgren U Melander A Ahren B & Burger A Stimulation of peripheral T_3 formation by oral but not by intravenous glucose administration in fasted subjects Acta Endocrinol 85 526 1977
30 Wilber J F & Utiger R D The effect of glucocorticoids on thyrotropin secretion J Clin Invest 48 2096 1969
31 Zingg W & Perry W F The influence of adrenal and gonadal steroids on the uptake of iodine by the thyroid gland J Clin Endocrinol Metab 13 712 1952





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A Simple Test for Autonomic Neuropathy in Juvenile Diabetics

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ABSTRACT The reproducibility of a simple method for determining the beat to beat variation during maximal respiration (duration of inspiration = duration of expiration = 5 sec), using an ordinary ECG apparatus and a ruler, was tested in 10 normals and 40 insulin treated diabetics The results were reproducible and beat to beat variation was found to be independent of a 25 minute period of rest The method was subsequently applied to 126 insulin treated diabetics and the results are in accordance with previous investigations on cardiac vagal neuropathy The method is applicable as a clinical routine test for cardiac vagal neuropathy

Key words autonomic neuropathy long term diabetes respiratory sinus arrhythmia

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In normals the respiratory sinus arrhythmia—the beat to-beat variation—generally exceeds 10/min decreasing after the age of 50 Recent reports show that long term diabetics have a reduced beat to-beat variation compared to age adjusted normals (2 8 10 12) These studies which included continuous registration of the cardiac electrical activity during normal (8) or forced respiration (10) required complicated electronic equipment

In this paper a simple method for measuring beat to beat variation is reported along with results from such measurements in a large insulin treated population

STUDY POPULATIONS

I *Evaluation of method* Ten normals (5 men and 5 women mean age 36 years S D 6) without familial disposition for diabetes mellitus and 40 insulin treated diabetics were tested Patients as well as normals were aged 20–40 years The patients who had no signs of diseases of the heart or lungs were selected at random among patients who fulfilled certain criteria of duration of diabetes and occurrence of peripheral neuropathy

Peripheral neuropathy was considered to be present if at least one of the following criteria was fulfilled 1) Absence of ankle and/or knee jerks 2) Decreased sense of vibration determined with a Bio-Thesiometer (9) 3) Paresthesias over a period of at least three months prior to the investigation None of the patients had orthostatic complaints

The diabetics were divided into four groups of ten 5 men and 5 women in each Group 1 Duration of diabetes 0–5 years no neuropathy Group 2 Duration of diabetes 6–15 years no neuropathy Group 3 Duration of diabetes >15 years no neuropathy Group 4 Duration of diabetes >15 years neuropathy

II *Measurements in insulin treated diabetics* One hundred and twenty six patients were selected at random among insulin treated outpatients Their age and sex distribution is given in Table I Mean age was 36 years duration of diabetes 3 months to 41 years There were no alcoholics among the patients and no patient received medication other than insulin The patients had no signs of diseases of the heart or lungs Forty eight patients had peripheral neuropathy as defined above Seven patients had symptomatic autonomic neuropathy i e diabetic diarrhea urinary retention or symptomatic orthostatic hypotension (decrease in systolic BP of more than 30 mmHg) verified in departments of gastroenterology or urology

METHODS

Experimental procedure No examination was carried out without the informed consent of the patient The patients were lying on a couch throughout the experimental procedures After explaining the procedures to the patient (5 min) ECG 1 was recorded during normal as well as forced (maximal) respiration (5 successive respiratory cycles) ECG 1 was recorded with duration of inspiration in the individual respiratory cycle = duration of expiration = 5 sec The recording was repeated 20 min later (ECG 2) ECG 3 was measured 5 min after ECG 2 during maximal respiration only The ECGs were recorded by a Mingograf

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Table I *Age and sex distribution of 126 insulin treated ambulatory patients*

Age (y)	Men	Women
26–30	16	17
31–35	15	19
36–40	10	12
41–45	21	16

Minor 3 ECG apparatus (evaluation of method) and a Cardioline Eta 1 ECG apparatus (measurements in insulin-treated diabetics) with 3 extremity leads and at a paper speed of 50 mm/sec In part II of the investigation only ECG 1 was recorded

Beat to-beat variation was determined according to Wheeler and Watkins (12) R–R intervals recorded during maximal respiration were measured with a ruler and the beat to-beat variation in the individual respiratory cycle was calculated as the difference between maximal and minimal heart rate and expressed as the mean of differences in 5 successive respirations

Statistical methods The patient groups were compared by means of the rank sum test for unpaired data and the χ^2 test Lowest level of significance was $p=0\ 05$ Correlation analyses were made by means of Spearman s rank coefficient correlation test

RESULTS

I *Evaluation of method* No significant difference found between beat to-beat variation during ECGs 1 2 and 3 in patient groups 1 3 and 4 and normals In group 2 beat to-beat variation was higher during ECG 1 than during ECG 3 while no significant differences were found during ECGs 1 and 2 and during ECGs 2 and 3 Mean differences in beat to-beat variation between ECG 1 and ECG 3 were +1 6 min^{-1} (range −5–+9) in normals +2 7 (range −2–+10) in group 1 +3 0 (range 0–+7) in group 2 +1 4 (range −3–+9) in group 3 and +0 3 (range −8–+11) in group 4

Testing beat to-beat variation during ECG 1 in men against women in all patient groups and normals yielded no differences by sex A significant inverse correlation was found between duration of diabetes and beat to-beat variation (ECG 1) ($R=-0\ 44$ $p<0\ 01$) and between age and beat to-beat variation in diabetics ($R=-0\ 41$ $p<0\ 02$) but not in normals

No significant correlation was demonstrable between resting heart rate and beat to-beat variation neither in patients nor in normals Furthermore no correlation was found between resting heart rate and beat to-beat variation in patients with reduced beat to-beat variation (<10/min)

Comparison between beat to-beat variation in the 4 patient groups and in the normals revealed no significant difference between normals and groups 1 2 and 3 However group 4 differed significantly from groups 1 2 and 3 as well as from the normals

II *Measurements in insulin treated diabetics* Out of the 126 patients 51 (40%) had reduced beat to-beat variation (<10/min) Patients with duration of diabetes longer than 15 years showed a significantly higher frequency of reduced beat to-beat variation than those with a shorter duration

Out of 48 patients (mean age 37 years S D 6) with peripheral neuropathy 34 (71%) had reduced beat to-beat variation against 6 of 7 patients (mean age 35 years S D 6) with symptomatic autonomic neuropathy Eleven (15%) of the 71 patients (mean age 35 years S D 6) without neuropathy had reduced beat to-beat variation (Fig 1) A significant difference was demonstrable between the prevalence of reduced beat to-beat variation in patients

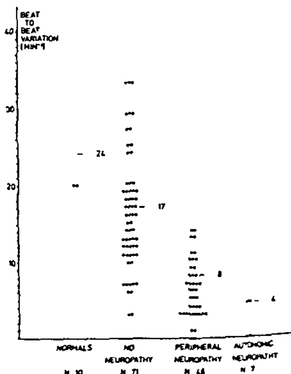


Fig 1 Beat to-beat variation in normal subjects and patients without neuropathy with peripheral neuropathy or symptomatic autonomic neuropathy

with peripheral neuropathy and in patients without neuropathy ($p<0.001$). No such difference was found between patients with peripheral and autonomic neuropathy.

Thirty three patients had a decreased sense of vibration and 22 of them had reduced beat-to-beat variation. Of 93 patients with a normal sense of vibration, 29 had reduced beat-to-beat variation. Thus the prevalence of reduced beat-to-beat variation was significantly higher in patients with a decreased than with a normal sense of vibration ($p<0.001$). A significant correlation was found between beat-to-beat variation (min^{-1}) and sense of vibration (V) ($n=126$, $R=-0.32$, $p<0.001$).

Twelve patients had a beat-to-beat variation of 9 or 10/min. In 8 of them the variation during the first respiration was significantly greater than the mean of the 4 subsequent respirations.

DISCUSSION

Respiration is the most important stimulus for sinus arrhythmia (3) and changes in depth and frequency of respiration alter the beat-to-beat variation (1). Thoracic stretch receptors are probably responsible for this phenomenon (4). A uniform pattern of respiration is therefore essential for a quantitation of respiratory sinus arrhythmia. The ideal would accordingly be to perform spirometry or pneumotachography simultaneously with ECG registration or measurement of changes in thoracic circumference (1). However, this is too time consuming for clinical routine purposes. Our results suggest that a fixed time interval of 5 sec for inspiration as well as expiration, along with maximal voluntary depth of respiration, is sufficient for obtaining reproducible results. Furthermore, the beat-to-beat variation was independent of a 25 minute period of rest, which increases the applicability of the method to clinical routine. The accuracy of paper speed of both ECG apparatuses was ±½%, which will not influence the size of beat-to-beat variation.

Our results are in accordance with previous reports on the prevalence of reduced beat-to-beat variation in diabetics (10) and also with the concept that reduced beat-to-beat variation is related to long term diabetes (8).

Out of 126 patients, only 7 (6%) had symptomatic autonomic neuropathy while 51 (40%) had reduced beat-to-beat variation. This finding is consistent with results on diabetic cystopathy, where abnormal urodynamic results were more frequent than clinical signs and subjective symptoms of cystopathy (7).

Reduced beat-to-beat variation was significantly more frequent in patients with peripheral neuropathy than in patients without neuropathy. This finding is in accordance with those by Ewing et al. (5), showing a dysfunction of the autonomic and peripheral nervous system to be parallel in long term diabetes.

Reduced beat-to-beat variation is considered a reliable sign of cardiac vagal neuropathy (2, 8, 10, 12). A new method for determining cardiac vagal neuropathy was published recently (6). It consists in determining the change in heart rate on changing from supine to standing position. The authors found that this heart rate response was reduced in diabetics with autonomic neuropathy. However, all their patients had orthostatic hypotension, which in itself increases the probability of an abnormal heart rate response to a change of position. Furthermore, the role of the sympathetic nervous system in this response is still unclear (2, 11). Thus, the two methods need to be compared in order to establish whether they give the same results in a diabetic population.

REFERENCES

1. Angelone, A. & Coulter, N. A.: J Appl Physiol 19: 479, 1964.
2. Bennett, T., Hosking, J. J. & Hampton, J. R.: Br Med J 2: 585, 1975.
3. Clynes, M.: Science 131: 300, 1960.
4. Davies, C. T. M. & Nielson, J. M. M.: J Appl Physiol 22: 947, 1967.
5. Ewing, D. J., Burt, I. R., Williams, I. W. et al.: J Neurol Neurosurg Psychiatry 39: 453, 1976.
6. Ewing, D. J., Campbell, I. W., Murray, A. et al.: Br Med J 1: 145, 1978.
7. Frimodt-Møller, O.: Dan Med Bull 23: 257, 1976.
8. Gundersen, H. J. G. & Neubauer, B.: Diabetologia 13: 137, 1977.
9. Jersild, M. & Lauritzen, E.: Le Diabete 6: 237, 1957.
10. Lloyd-Mostyn, R. H. & Watkins, P. J.: Br Med J 3: 15, 1975.
11. Page, M. McB. & Watkins, P. J.: Clin Endocrinol Metab 6: 377, 1977.
12. Wheeler, T. & Watkins, P. J.: Br Med J 4: 584, 1973.

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Effect of Clinical Bed Rest for Seven Days on Physical Performance

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ABSTRACT Twenty two healthy men were confined to bed in a hospital ward (clinical bed rest) for one week Variables related to physical fitness were measured before and immediately after the bed rest period, as well as one and three months thereafter As a result of bed rest, maximal oxygen uptake $\dot{V}_{O_2}$ and total Hb decreased to 94%, and calculated blood volume to 93% of the initial value Hb concentration and Hct were unaltered Thus red cell and plasma volumes were proportionately reduced No significant orthostatic dysfunction developed The blood lactate peak at maximum work remained unchanged (14 0 mM/l) The resting excretion of noradrenaline decreased moderately during the bed rest period, whilst that of adrenaline was unchanged *Body weight decreased by a mean of 0 7* kg

Key words bed rest performance oxygen uptake blood volume tilt tolerance catecholamine excretion
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Adverse effects of confinement to bed were pointed out by Asher as early as 1947 (2) There is now ample documentation that physical fitness deteriorates with prolonged bed rest (6 8 9 19)

To what extent bed rest induced physical impairment occurs in hospital patients appears to be less well investigated In available clinical studies many of which have been performed in surgical departments the operation trauma in the investigated patients might have influenced results (1 4 7) or longer lasting or stricter bed rest regimes have been used (8 21) than those currently practised in most hospital wards for acutely ill patients (11 12)

The present investigation was undertaken to study the influence of a hospital bed rest regime (clinical bed rest) on some variables related to physical fitness This knowledge is necessary in order to evaluate similar studies of various disease states in hospital patients

The period of bed rest (one week) was chosen to correspond to the mean bed rest time of those patients of a similar age group who were admitted to the department for various acute infectious diseases during the period of the study (11) Young patients were selected to avoid the influence of complicating cardiovascular disorders and for practical and statistical reasons only men were included

SUBJECTS

Twenty-two healthy men the majority of whom were *students aged 25 0±0 6 years were confined to bed for* seven days one at a time in a special room in a hospital ward for infectious diseases They followed the same daily routine as the patients of the ward Thus they were allowed up only for personal hygiene and sat in an arm chair for 15 min twice daily on the 5–7th days Since patients suffering infectious diseases eat less while febrile (11) the energy intake of six subjects was reduced to 2 000 kJ a day during the first four days and thereafter increased to 9 200 kJ a day i e the normal amount of energy served to the patients Fluid intake was unlimited Since many patients are given antipyretic drugs when febrile mostly acetyl salicylic acid that drug was given to another six subjects in a dose of 3 g daily

PROCEDURE AND METHODS

Measurements were performed on four occasions one week prior to bed rest immediately after bed rest one month later and three months after bed rest Measures were taken to avoid a training effect as a result of the programme (11)

On each occasion the following measurements were made body weight total Hb Hb concentration orthostatic tolerance physical working capacity blood lactate

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Table I *Maximal oxygen uptake ($V_{O_2 max}$) maximal ventilatory volume ($V_{E max}$) total Hb Hb concentration blood volume (BV) red cell volume (RCV) and plasma volume (PV) before and after clinical bed rest for seven days and one and three months thereafter*

** ($p<0 01$) and *** ($p<0 001$) denote a statistically significant difference when compared to the pre bed rest value

	Before bed rest			After bed rest			1 month control			3 month control		
	$\bar{x}$	S D	N	$\bar{x}$	S D	N	$\bar{x}$	S D	N	$\bar{x}$	S D	N
$V_{O_2 max}$ (l×min⁻¹ STPD)	3 30	0 46	22	3 11**	0 39	21	3 33	0 49	22	3 37	0 43	21
$V_{E max}$ (l×min⁻¹ STPD)	93 0	16 3	22	90 9	15 9	21	94 4	14 7	22	96 9	14 2	21
Total Hb (g)	695 6	74 4	22	653 2***	72 1	22	677 5	73 9	22	688 8	70 7	21
Hb concentration (g×100 ml⁻¹)	14 8	0 9	22	14 9	0 8	22	14 6	0 8	22	14 8	0 8	21
BV (l)	5 16	0 51	22	4 82***	0 43	22	5 10	0 47	22	5 15	0 60	21
RCV (l)	2 06	0 22	22	1 93***	0 21	22	2 01	0 22	22	2 04	0 21	21
PV (l)	3 10	0 34	22	2 88***	0 27	22	3 12	0 35	22	3 11	0 43	21

concentration during exercise and catecholamine excretion during rest and exercise

Total Hb was determined using the indirect CO method according to Sjöstrand (20) Total blood volume was calculated using Hb concentration Red cell volume and plasma volume were then calculated using a Hct/Hb ratio of 2 96 Hct was actually determined in the last 103 measurements of the present study confirming this ratio to be accurate and constant Blood was sampled from a cubital vein without stasis the measured Hb and Hct values being corrected (×0 91) before use in calculations Blood volume determinations were always performed in duplicate at an interval of one day and the mean value was used The coefficient of variation for the single determination was 3 3%

The orthostatic tests were also performed on two consecutive days using a tilt board tilt angle 70 Heart rate was measured from ECG in horizontal position after at least 10 min rest or until stable recordings were obtained and then each minute during 8 min of tilting using the maximal value in the calculations There was no significant difference between the results of the duplicate tilt board tests the last results are presented here

The exercise tests were performed on a bicycle ergometer and carried out until exhaustion They included direct measurements of maximal oxygen uptake (3) On the highest load gas collection in the Douglas bag had sometimes to be commenced after only 1–2 min of work Exercise data are expressed as W_{170} and as amount of performed work $W_{max\ perf}$ (22)

For peak lactate determination arterialized blood was sampled from the finger tip within 3 min after interruption of exercise Lactate was determined according to Hohorst (13) As calculated by analysis of variance the errors of sampling and analysis were 0 41 and 0 16 mmol/l respectively at a mean of 12 97 mmol/l

Urine was collected during the test period The collection started immediately prior to the test when the subject urinated and was interrupted as soon as it was possible for the subject to empty his bladder after the end of the test Thus the urine sampling time included both rest (mean 52 min) and exercise (mean 30 min) the proportion of rest to exercise being the same on all occasions of measurements

The blood volume determinations and tilt board tests were performed in the morning before breakfast and the exercise tests at 1 p m after a light meal at about 11 a m

During bed rest all urine was collected during days 1 5 and 6 The day and night urines were collected separately the break points being 7 a m and 10 p m Determinations of noradrenaline and adrenaline in the urine were carried out according to Euler and Lishajko (10)

Catecholamine determinations were performed in duplicate in each of two separate urine samples the coefficients of variation for the single determination (calculated from the mean value of each sample) being 4.5% for noradrenaline and 12 0% for adrenaline

Comparisons were made using Student s *t* test for paired observations

RESULTS

Results are given in Table I and Figs 1 2 and 3

As a result of clinical bed rest for one week the maximal oxygen uptake was significantly reduced to 94 2% of the initial value Similarly W_{170} and $W_{max\ perf}$ were reduced to 93 8 and 96 3% respectively (Table I, Fig 1) No correlation was found between initial performance and extent of deterioration

The ventilatory volume (Table I) and ventilatory coefficient at maximal exercise were not significantly influenced the mean value of the latter for all four occasions being 28 6±0 4 Likewise the maximal heart rate did not change after bed rest (mean 195 0±0 8 beats/min)

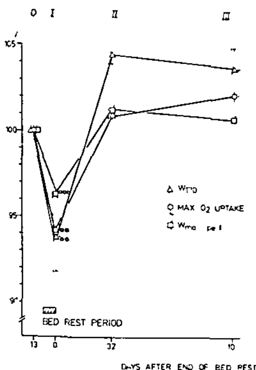


Fig. 1. W_{170}, maximal oxygen uptake and $W_{max\ perf}$ in 22 healthy men before (0) and after (*I*) clinical bed rest for seven days and one (*II*) and three (*III*) months thereafter (mean±S.E.M. of mean pre bed rest recordings). ** ($p<0.01$) and *** ($p<0.001$) denote a statistically significant difference when compared to the pre bed rest value.

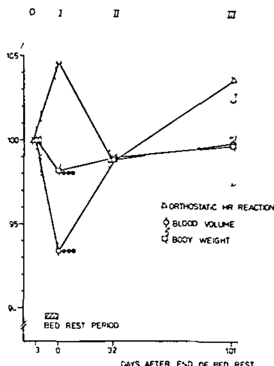


Fig. 2. Orthostatic heart rate (*HR*) reaction, blood volume and body weight. Symbols as in Fig. 1.

Excretions of noradrenaline and adrenaline during work and peak blood lactate concentration were the same on all occasions of measurement, indicating that the tests were maximal (mean excretion of noradrenaline 40.6±1.6 and of adrenaline 9.9±0.7 ng/min, mean peak lactate concentration 14.0±0.4 mM/l).

Neither were the resting heart rate (mean 74±2.9 beats/min) and the maximal heart rate during tilting (Fig. 2) significantly altered by one week's rest in bed. Similar non significant results were obtained when calculations were based on the individual differences between resting and maximal heart rate recordings.

The total Hb was reduced to 93.9 and the calculated blood volume to 93.4% of the original values. The relative contribution of red cell mass and plasma volume to blood volume contraction was proportionate to their original volumes, since Hb concentration was unaltered (Table I, Fig. 2). There was no correlation between calculated change in plasma volume and change in orthostatic heart rate reaction (correlation coefficient=0.30). Body weight decreased by a mean of 0.7 kg (Fig. 2).

After one month all measured variables had resumed their initial values, although total Hb still tended to be lower ($t=1.89$).

During clinical bed rest a moderate reduction of the resting noradrenaline excretion occurred, whilst that of adrenaline was unchanged (Fig. 3).

No differences in any of the measured variables were found between the six subjects who were on a low-energy diet, or those who received acetyl salicylic acid and the others.

DISCUSSION

In the present study, the recorded decrease in physical performance after one week's clinical bed rest was moderate but statistically secured when expressed as W_{170} or $W_{max\ perf}$ or when directly measured as maximal oxygen uptake. A similar limited performance reduction to 94–96% of the initial

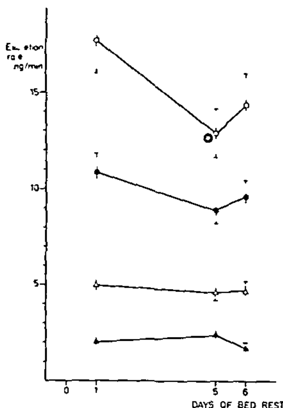


Fig 3 Excretion rates of noradrenaline (O ●) and adrenaline (Δ ▲) during days 1 5 and 6 of clinical bed rest Open symbols denote values in day urines filled symbols in night urines (mean ± S E M) ($p<0.05$) denotes a statistically significant difference when compared to the value on day 1

value was recorded by Adolfsson (1) and Carswell (7) in surgical patients one week after the operation although the operation trauma evidently did cause an additional deteriorating effect since Carswell recorded considerably lower values four days post operatively In Adolfsson s and Carswell s studies however the bed rest time was only 1–3 days

In order to evaluate the possible effects of clinical bed rest on cardiovascular responses to exercise Bassey et al (4) working with orthopaedic patients tried to eliminate the influence of the operation trauma and therefore investigated patients who had undergone meniscectomy supposing that minor operations have negligible effects on the central circulatory regulation However after only 4 days bed rest following the operation their patients heart rate response to exercise at an oxygen uptake of 1 2 l/min had increased by 23 beats/min compared with 16 beats after 7 days bed rest in the present study Although the authors were of a different opinion this might indicate an additional effect of the operation trauma or differences in the bed rest regimes providing their small study group (5 patients) is representative

A positive correlation between initial performance and extent of deterioration during bed rest as shown by previous authors (1 5) could not be established in the present study This might have been due to conformity in initial training level within the study group

Since immobility may induce a shunting effect due to fluid shifts within the lungs (18) breathing work might be expected to be higher after bed rest However in the present study ventilatory efficiency was not reduced after bed rest the ventilatory volume during maximal work after bed rest corresponded to the maximal oxygen uptake Similar results were recorded by Saltin et al (19) after three weeks immobilization

The present finding of an unchanged maximal heart rate after bed rest accords with previous investigations (19) but some authors have recorded an increase (23)

The collection time of urine at the exercise tests included both work and rest The recorded excretion rates of catecholamines were of the same order of magnitude as those found by Holmgren (14) Although the levelling-off criterion of oxygen uptake was not fulfilled in all the exercise tests the very high peak lactate concentrations would insure that the tests were performed to maximum (3)

The finding of a decreased derived plasma volume after one week s bed rest is in accordance with previous short term bed rest studies in fact a significant plasma volume reduction has been recorded after only 12 hours rest in bed (24) The significant decrease in total Hb and in calculated red cell volume was somewhat less expected however Such a reduction is well documented after prolonged bed rest (9 15 17) while after about one week s confinement to bed no consistent change has been recorded in previous studies (17) However the reductions in the present investigation were small Therefore the lack of a significant change in earlier studies might be explained by differences in methods or in sizes and composition of study groups

One might have expected a correlation of decrease in plasma volume to orthostatic deterioration since many authors consider decrease in plasma volume to be the main factor causing short

term bed rest deconditioning (4 8) Although a trend was recorded no significant orthostatic dysfunction developed in the present subjects the allowed activity level probably being sufficiently preventive

The moderate reduction of the excretion rate of noradrenaline during the course of bed rest while that of adrenaline was unchanged is in accordance with earlier investigations (16)

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REFERENCES

1 Adolfsson G Circulatory and respiratory function in relation to physical activity in female patients before and after cholecystectomy Acta Chir Scand (Suppl) 401 1969

2 Asher R A J The dangers of going to bed Br Med J 2 967 1947

3 Åstrand P O Experimental studies of physical working capacity in relation to sex and age Diss Med Karolinska Institutet Stockholm Munksgaard Copenhagen 1952

4 Bassey E J Bennett T Birmingham A T Fentem P H Fitton D & Goldsmith R Effects of surgical operation and bed rest on cardiovascular responses to exercise in hospital patients Cardiovasc Res 7 588 1973

5 Bassey E J & Fentem P H Extent of deterioration in physical condition during postoperative bed rest and its reversal by rehabilitation Br Med J 4 194 1974

6 Birkhead N C Blizzard J J Daly J W Haupt G J Issekuts B Jr Myers R N & Rodahl K Cardiodynamic and metabolic effects of prolonged bed rest NASA Technical Documentary Report no AMRL TDR-63 37 May 1963

7 Carswell S Changes in aerobic power in patients undergoing elective surgery J Physiol (Lond) 251 42P 1975

8 Chobanian A W Lille R O Tercyak M A & Blevins P The metabolic and hemodynamic effects of prolonged bed rest in normal subjects Circulation 49 551 1974

9 Deitrick J E Whedon G D & Shorr E Effects of immobilization upon various metabolic and physiologic functions of normal men Am J Med 4 3 1948

10 Euler U S & Lishajko F Improved technique for the fluorometric estimation of catecholamines Acta Physiol Scand 51 348 1961

11 Friman G Effects of acute infectious disease on circulatory function Acta Med Scand (Suppl) 592 1976

12 Haggmark T A study of morphologic and enzymatic properties of the skeletal muscles after injuries and immobilization in man p 9 Thesis Karolinska Institutet Stockholm 1978

13 Hohorst H J L (+) Lactat Bestimmung mit Lactatdehydrogenase und DPN In Methoden der enzymatischen Analyse (ed H V Bergmeyer) p 266 Verlag Chemie Weinheim 1962

14 Holmgren A Circulatory changes during muscular work in man Scand J Clin Lab Invest (Suppl) 24 1956

15 Lancaster M C Hematologic aspects of bed rest In Hypogravic and hypodynamic environments (ed R H Murray & M McCally) p 299 NASA SP 269 Washington D C 1971

16 Leach C S Hulley S B Rambant P C & Dietlein L F The effect of bed rest on adrenal function Space Life Sciences 4 415 1973

17 Miller P B Johnson R L & Lamb L E Effects of four weeks of absolute bed rest on circulatory functions in man Aerospace Med 35 1194 1964

18 Ray J F III Yost L Moallem S Sanoudus G M Villamena P Paredes R M & Clauss R H Immobility hypoxemia and pulmonary arteriovenous shunting Arch Surg 109 537 1974

19 Saltin B Blomqvist G Mitchell J H Johnson R L Jr Wildenthal K & Chapman C B Response to exercise after bed rest and after training Circulation (Suppl) 7 1968

20 Sjostrand T A method for the determination of the total haemoglobin content of the body Acta Physiol Scand 16 211 1948

21 Sokoll U Kessel R & Lang E Auswirkungen einer langeren Immobilisation auf die Herz und Kreislaufsdynamik Munch Med Wochenschr 115 69 1973

22 Strandell T Heart rate arterial lactate concentration and oxygen uptake during exercise in old men compared with young men Acta Physiol Scand 60 197 1964

23 Stremel R W Convertino V A Bernauer E M & Greenleaf J E Cardiorespiratory deconditioning with static and dynamic leg exercise during bed rest J Appl Physiol 41 905 1976

24 Vogt F B Tilt table and plasma volume changes with short term deconditioning experiments Aerospace Med 38 564 1967

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Self-Therapy for Haemophilia in Norway

Effect on Transfusion Frequency and Days Lost from Work

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ABSTRACT During the years 1975–77, 35 patients with haemophilia A or B (factor level of 1% or less) were instructed to administer concentrates of the deficient factor (mean dose 14 U/kg) i v in case of episodic uncomplicated bleedings without prior consultation with a physician At Dec 1977, 33 of these patients (29 with haemophilia A 4 with B) were taking part in the program They represent 35% of all Norwegian patients above 7 years of age with severe haemophilia in whom high titered antibodies have not been demonstrated For 13 patients records from 2 years on self therapy have been compared with records from the last year before self therapy During the first year on self therapy the number of days lost from work dropped by 77%, while the number of transfusions increased by 22% During the second year, days lost from work were still low (68% decrease compared to the year preceding self therapy) and the transfusion frequency remained unchanged Significant side effects of antihaemophilic concentrates were not observed and vein damage was not a problem Joint motion studies did not indicate progress of haemophilic arthropathy during self therapy Properly supervised self therapy is practical and safe and improves considerably the quality of life

Key words haemophilia self medication
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It is generally accepted that in patients suffering from haemophilia early replacement therapy reduces the pain and joint damage induced by acute bleedings For this reason patients with severe haemophilia A or B have been offered training in self therapy at the Institute for Haemophilia in Norway since 1975 Home treatment of haemophilia has been tried out in several countries during the last decade (8 9 11 12) In this paper we present our experiences with this mode of treatment A detailed survey of the principles and training applied in our program has appeared earlier (2) Preliminary experiences have been published in abstract form (4)

PATIENTS AND METHODS

Patients From May 1975 to Dec 1977 35 patients with haemophilia A or B participated in the Norwegian home transfusion program Two patients have been permanently excluded leaving a total of 33 patients (Table I) All the patients had factor VIII or IX levels of ≤1% of average normal Four patients had non anamnestic low-titered antibodies and were classified as low responders according to the criteria set up by Allain and Frommel (1) The treatment of these patients and of the others did not differ The patients were strongly encouraged to administer the treatment themselves and all but 4 do so Two patients are treated by their wives and 2 brothers (aged 6 and 7 years at the start of training) are treated by their parents Therefore our home therapy program is essentially a self therapy program and we have chosen that term in this paper

Criteria for selection of patients The patient should bleed at least once every month or be living far from a treatment center He should have good veins and not have anamnestic high titered antibodies directed against factor VIII or IX We have not excluded patients during the training course because of a lack of the necessary emotional stability but some patients were not invited to participate for this reason

Training for self therapy The patients were given theoretical and practical training often in pairs at the Institute for Haemophilia in Oslo by two nurses and a doctor If the patient was a child his parents were also instructed but if at all feasible the boy was strongly encouraged to treat himself As soon as the patient was competent usually after a week s intensive training he was given a self therapy certificate and a detailed manual (3) (copies of which were sent to the treating physician or hospital) together with 5 self therapy packs The equipment has been described previously (2 3) Coagulation factor concentrates were given as freeze-dried cryoprecipitate or factor IX concentrate produced either by the Blood Bank and the Department of Immunohaematology Ullevål Hospital or the Finnish Red Cross

Table V *Haemophilic patients with elbow flexion deformities of ≥20° and knee flexion deformities of ≥10° before start of self therapy*

Arthrodesis of the knee joint had been performed in 2 patients

Age (y)	Right Elbow	Right Knee	Left Elbow	Left Knee
<10	0/3	0/3	0/3	0/3
10–20	3/8	1/8	3/8	2/8
21–30	9/15	1/15	7/15	1/15
>30	5/7	4/7	6/7	3/7
	17/33	6/33	16/33	6/33

HB_sAg/Ab status and liver chemistries

Table IV shows that only one patient was HB_sAg positive at the start of self therapy but 51 5% had antibodies directed against this antigen Serum bilirubin was normal in all cases increased levels of ASAT or ALAT were not correlated with the presence of HB Ab (Table IV) During self therapy only one patient is known to have been icteric so far He is now anicteric again and in good health Three out of 13 patients developed abnormal levels of transaminases after 2 years on self therapy The long term significance of such findings is not known at present

Musculoskeletal evaluation

Joint motion studies were carried out before and 2 yearly after self therapy to assess the development of haemophilic arthropathy As expected significant flexion deformities were positively correlated with increasing age (Table V) The patients were encouraged to exercise and take part in recreational activities and if flexion deformities existed the patient was referred to a physiotherapist Table VI shows that the haemophilic arthropathy did not appear to progress during the next 2 years It must be admitted however, that the observation time is short for these parameters

DISCUSSION

Self administration of coagulation factor concentrates by patients with haemophilia is a recent development which has been very favorably accepted by patients Initially, the attitude among doctors and other health personnel was skeptical but the very few medical complications the enthusiasm of the patients and the time saved for busy hospital staffs have made self therapy a preferred mode of treatment among them too More than 35% of all Norwegian patients above 7 years with severe haemophilia in whom high titered antibodies have not been demonstrated, are now included in the program and the figure is likely to increase However not all patients are eligible for self therapy Firstly the patient and his family must want it Secondly, they must have sufficient emotional stability to accept the responsibility Thirdly there is an age limit below which home therapy is inadvisable even when administered by skilled parents because of increased difficulties with venipuncture assessment of the site of the bleeding and its severity and possible psychological hazards

The first dozen patients we trained were selected and thus it is no surprise that training of those who followed has been more demanding However our overall experience is that lay people manage this mode of therapy remarkably well provided they get

Table VI *Flexion deformities in elbows and knees before and after 2 years on self therapy in 13 patients with haemophilia A or B*

	Right Before	Right After 2 years self-therapy	Left Before	Left After 2 years self-therapy
No of pats with elbow flexion deformity	7	7	8	8
≥20	6	4	7	4
No of pats with knee flexion deformity	7	7	5	5
≥10	5	5	2	2
Mean circumference of the thigh 15 cm above the knee* (cm)	37 2	37 5	36 7	37 3

* Measured in 9 patients

proper training and follow up Our initial training period of approximately one week may appear short It reflects the circumstance that many of our patients were well informed about their disease and its treatment in advance through their doctor and by participation in summer camps which have been arranged in Norway since 1969

Side-effects of the treatment have been few and insignificant We consider freeze-dried cryoprecipitate a safe and suitable preparation for self therapy and prefer it to commercial concentrates because of its considerably lower cost and because it means better utilization of national blood resources Evidence of hepatitis in relatives was not observed and thus did not pose a problem relevant to this mode of treatment The relatively high incidence of HB_sAb-positive patients (51 5%) only attests to the risk which multitransfused patients run The various figures reported ranging from 46% in an English group (10) to 90% in a group of American patients (5) may reflect methodological differences rather than true differences in incidence

Overuse of therapy has not been a problem The moderate increase in transfusion frequency observed so far is not unexpected Several of the patients admitted that before self therapy they would sometimes let bleedings subside without treatment rather than take a trip to the hospital and wait for therapy in busy wards We are more concerned about the increased utilization of blood products (Table III) which is partly due to an increase in the dose per bleeding while on self therapy Other treatment centers have reported that introduction of home therapy causes either little difference (8 11) or moderately increased usage (7) Thus we must consider the possibility of reducing our dose of 14 U/kg In Oxford the standard home treatment dose is 250 U or approximately 4–5 U/kg and this proved satisfactory in resolving the majority of bleeds (12)

The most dramatic hard data collected in this project is the substantial decrease in days lost from school and work which also has been reported by others (6 9 11) One important contributory factor is that bleedings are treated so early that pains are rapidly relieved and function can be quickly resumed Another is the psychological impact of aquiring useful independence increased self regard and normalization of the life pattern It is here that the most important outcome of the program is to be found but this cannot be expressed in figures Significantly no one has desired a return to the former method of hospital treatment However enthusiasm for self therapy should not blind the physician to the hazards of this approach particularly if the doctor/patient relationship suffers damage and treatment is not followed up meticulously

There is also an economic aspect to self therapy It has been claimed to represent a saving (9) mainly because of reduced hospital and doctor fees A cost/benefit analysis of our program is in progress

REFERENCES

1 Allain J P & Frommel D Antibodies to factor VIII V Patterns of immune response to factor VIII in haemophilia A Blood 47 973 1976
2 Evensen S A Home transfusion for haemophilia Tidsskr Nor Laegeforen 96 687 1976
3 Evensen S A Thaule R & Grøan K Hjemme transfusjon for blødersykdom Praktisk veiledning for pasienter Institutt for blødere Oslo 1977
4 — Home therapy for haemophilia in Norway effect on transfusion frequency and absenteeism Thromb Haemostas 38 368 1977
5 Hilgartner M W & Giardina P Liver dysfunction in patients with hemophilia Scand J Haematol (Suppl) 30 6 1977
6 Lazerson J Hemophilia home transfusion program effect on school attendance J Pediatr 81 330 1972
7 — Hemophilia home transfusion program effect on cryoprecipitate utilization J Pediatr 82 857 1973
8 Le Quesne B Britten M Maragaki C & Dormandy K M Home treatment for patients with haemophilia Lancet 2 507 1974
9 Levine P H & Britten A F H Supervised patient management of hemophilia A study of 45 patients with hemophilia A and B Ann Intern Med 78 195 1973
10 Levine P H McVerry B A Attock B & Dormandy K M Health of the intensively treated hemophiliac with special reference to abnormal liver chemistries and splenomegaly Blood 50 1 1977
11 Rabiner S F Telfer M C & Fajardo R Home transfusion of hemophiliacs JAMA 221 885 1972
12 Rizza C R & Spooner R J D Home treatment of haemophilia and Christmas disease five years experience Br J Haematol 37 53 1977

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Biochemical Variables Related to Calcium Metabolism in Epileptics

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ABSTRACT Five biochemical variables, S Ca, U Ca, S-P, U P and S ALP, all involved in calcium metabolism, have been investigated in 86 epileptics on long term medication We found hypocalciuria in half of the epileptics and increased S-ALP in one third In contrast to earlier reports there was no hypocalcemia, whereas hypercalcemia was found in 7 epileptics We have previously reported a high frequency of fractures in these epileptics An increased fracture rate was found in the 13 epileptics with both hypocalciuria and increased S-ALP, indicating osteomalacia

Key words epilepsia anticonvulsant drugs osteomalacia fragility fractures

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Disturbances of the calcium metabolism in epileptics on anticonvulsant drugs so-called anticonvulsant osteomalacia has been frequently reported during the last decade (5 6 7 8 9 11 13 17 21 22)

In a group of epileptics the incidence of non seizure related fractures was found to be six times higher than expected (12) This group has now been studied with regard to biochemical variables related to calcium metabolism

CLINICAL MATERIAL

The clinical material comprised 86 epileptics 48 women and 38 men with a mean age of 59±12 years Most of them had suffered from the disease since childhood and had been on anticonvulsant drugs for many years (median 49 years) The drugs most frequently used were diphenylhydantoin 100–500 mg daily and/or phenobarbital 100 mg daily Diphenylhydantoin was used in 53 cases as the only drug in 16 epileptics and phenobarbital in 51 Other drugs used were carbamazepine in 26 cases and primidone in 5 Six epileptics had no medication at the time of blood sampling but had been on medication for prolonged periods in the past In these cases the medication had been discontinued 2–8 years before the present investigation Serum diphenylhydantoin concentrations were not routinely determined The epileptics were debilitated to a minor degree and lived in a home for epileptics (Fogdarodshemmet) None were on steroid therapy two had diabetes mellitus and one rheumatoid arthritis

In 52 of these epileptics there was no history of fractures during the past seven years 21 had had 44 fractures unrelated to epileptic seizures and 15 had had 26 fractures related to epileptic seizures The mean age was not significantly different between epileptics without fractures epileptics with fractures related or not related to epileptic seizures

METHODS

The following variables were studied serum calcium (S-Ca) urinary calcium (U Ca) serum inorganic phosphate (S P) urinary inorganic phosphate (U P) and serum alkaline phosphatases (S-ALP) All analyses were made in triplicate by one and the same technician S-Ca and U Ca were determined by conventional flame photometry S-P and U P according to Chen et al (2) as acid solvent inorganic phosphate S-ALP was determined as an enzyme activity where serum acted on paranitrophenyl phosphate in the presence of alkali the amount of liberated paranitrophenyl being measured photometrically U creatinine was determined with a Technicon AutoAnalyzer

All blood samples were drawn and urine samples were collected and immediately acidified with HCl in the morning after an overnight fast Twenty four-hour urine samples could not be collected for practical reasons U Ca and U P have therefore been expressed in relation to creatinine excretion Reference values for U Ca and U creatinine have been computed from the reference values of the laboratory in Malmo assuming a standard body weight of 70 kg No reference values can be given for

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Abbreviations S-Ca = serum calcium U-Ca = urinary calcium S-P = serum inorganic phosphate U P = urinary inorganic phosphate S-ALP = serum alkaline phosphatase

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Table I *Biochemical variables in 86 epileptics (mean ± S D)*

Subset	N	S-Ca (mmol/l)	U Ca (mmol/mol creatinine)	S-ALP (μkat/l)	S-P (mmol/l)	U P (mmol/mol creatinine)
Men						
1	23	2 46±0 08	253±164	4 1±1 2	1 0±0 1	2 344± 679
2	7	2 46±0 10	263± 97	4 1±0 9	1 1±0 1	2 186± 381
3	8	2 50±0 09	296±132	3 8±0 7	1 1±0 2	2 328± 572
Women						
4	29	2 47±0 12	280±186	4 4±1 8	1 1±0 1	2 529± 572
5	14	2 48±0 10	372±308	6 1±4 1	1 1±0 1	2 833± 572
6	5	2 49±0 05	174±108	4 1±0 5	1 1±0 1	3 054±1 014
Reference value		2 2–2 6	♂ 198–497 ♀ 288–586	0 8–4 6	0 7–1 5	

U P since it is primarily dependent on dietary intake Since all the epileptics were institutionalized and shared the same meals a comparison of U P between individual epileptics was considered worthwhile

Statistical methods

For the statistical analysis performed by T Polfeldt Department of Mathematical Statistics University of Lund the cases were divided into six subsets 1) Men without fracture 2) Men with fracture unrelated to seizure 3) Men with fracture related to seizure 4 5 6) Similarly for women For each of the five variables studied and ages the mean values of the following subsets were compared 1 with 2 1 with 3 2 with 3 2 with 1 and 3 combined and similarly for women Furthermore the following six pairs of variables were plotted in scatter diagrams S-Ca and U Ca S-Ca and S-P S Ca and S-ALP U Ca and S-P U Ca and S-ALP S-P and S-ALP Different symbols were used for the subsets 1+3 2 4+6 5 A graphical two-dimensional analysis was thus performed Using only the subsets men (1+2+3) and women (4+5+6) correlation coefficients and linear regressions were calculated for the same pairs of variables

RESULTS

The statistical analysis could not demonstrate any significant differences in S Ca S-P S-ALP and U P between women and men or between the epileptics without fractures (subsets 1 and 4) with fractures unrelated to seizure (subsets 2 and 5) or seizure related fractures (subsets 3 and 6) (Table I)

Table II *Number of epileptics with laboratory values outside the normal values and the combinations of abnormal values*

No of epileptics with combinations of abnormal values	S-Ca >2 6 mmol/l (n=7)	U Ca (mmol/mol creatinine) > ♀ 586 ♂ 497 (n=9)	U Ca (mmol/mol creatinine) < ♀ 268 ♂ 198 (n=42)	S-ALP >4 6 μkat/l (n=30)	S-P >1 3 mmol/l (n=1)
2	××		××		
2	××		××	××	
1	×	×		×	
1	×			×	
1	×				
4		×× ××		×× ××	
4		×× ××			
26			26		
11			××××× ××××× ×	××××× ××××× ×	
1			×		×
11				××××× ××××× ×	

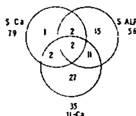


Fig 1 Combinations of hypercalcemia hypocalciuria and increased levels of S-ALP Numbers of individuals with values within normal levels are given outside the diagram

The individual samples falling outside the reference values are shown in Table II which also shows the combinations of abnormal values and as a Venn diagram in Fig 1 Seven epileptics were hypercalcemic four of whom demonstrated hypocalciuria and four increased S-ALP levels One of them had hypercalciuria Nine epileptics were hypercalciuric five of whom also had raised S ALP levels *Forty two demonstrated hypocalciuria 13 of* them in connection with increased S ALP and one in connection with raised S-P Finally 11 subjects demonstrated isolated increases in S ALP levels

The 13 epileptics with hypocalciuria and raised levels of ALP showed a significantly increased rate of fractures compared with the other epileptics with no less than 14 out of the 44 non seizure related fractures (0 5 $> p >$ 0 01)

DISCUSSION

Abnormal findings with regard to biochemical variables have been frequently demonstrated in epileptics on long term medication Studies have been carried out in ambulatory adults (17) and ambulatory children (7) as well as in non ambulatory adults (21) and non ambulatory children (9) These investigations report hypocalcemia in 7–30% and increased S-ALP in 24–43% of the epileptics In a recent report (17) hypocalciuria was found in 18% These changes are more severe in epileptics treated indoors as in most institutions as well as in those on combined diphenylhydantoin and phenobarbital therapy (7 13) There are however reports on epileptics without pathological deviations in biochemical variables (14 18) The latter study comprised both out and in patients but only six in each group There are several reports showing reduced calcium resorption from the small intestine in epileptics (10 15 20)

Cholecalciferol (vitamin D_3) is hydroxylated in the liver producing 25 hydroxy D_3 The next hydroxylation occurs in the kidney tubule producing inactive as well as active metabolites The active metabolite 1 25-dihydroxy D_3 affects the mucous membranes of the small intestine and increases calcium resorption from the gut Both Hahn et al (7) and Mosekilde et al (16) have shown low serum concentrations of 25 hydroxy D_3 in epileptics on anticonvulsant drugs The estimation of concentrations of the various vitamin D metabolites is however involved with great difficulties The interference which the antiepileptic drugs cause with vitamin D metabolism has still to be established

Like other researchers we found hypocalciuria and increased S-ALP in some epileptics But in contrast to previous reports we found no epileptics with hypocalcemia A possible explanation is that the daily intake of calcium and vitamin D in the subjects of this study is high enough to prevent the *development of hypocalcemia Another explanation* might be that these epileptics are more exposed to sunlight and physical activity than those in other reports (7 13) The effect of the sun could be the reason why no anticonvulsant osteomalacia was found in a report from Africa (1)

We have reported an increased incidence of fractures in these epileptics (12) However there were no statistically significant differences between epileptics with or without known skeletal fragility with regard to the biochemical findings except for the 13 epileptics with hypocalciuria and raised levels of ALP In an attempt to penetrate this issue further the individual determinations falling outside the reference values have been analyzed (Table II Fig 1) We found seven epileptics with hypercalcemia a number which is unexpectedly high Christensson et al (4) found 3 9% with single S-Ca levels above their reference level in a study of a large population in Stockholm The majority of these hypercalcemic patients have presumably hyperparathyroidism (3) If so the hypocalciuria found in four hypercalcemic epileptics could be a sign of renal damage and the increased S ALP found in four an indication of skeletal involvement There is no obvious reason why hyperparathyroidism should be more common in epileptics on anticonvulsant drugs One could speculate that so-called anticonvulsant osteomalacia elicits secondary hyperparathyroidism which later becomes autonomous If this were

true the four epileptics with hypercalciuria and raised S-ALP could represent epileptics with secondary hyperparathyroidism On the other hand there was not a single case of hypocalcemia which should have preceded the development of hyperparathyroidism

The epileptics with hypocalciuria and/or raised S-ALP levels are of particular interest They could in fact represent osteomalacia at a stage when hypocalcemia has not yet come into the open (19) It was therefore of particular interest to study the fracture epidemiology in these epileptics Their fracture rate was higher than that of the rest of the epileptics

REFERENCES

1 Apantaku J B Afonja O A & Boyo A E The effect of long term anticonvulsant therapy on serum calcium phosphate and alkaline phosphatase in Nigerian epileptic patients Trop Geogr Med 27 418 1975
2 Chen P S Toribara T Y & Warner H Microdetermination of phosphorus Anal Chem 28 1756 1956
3 Christensson T Hellstrom K & Wengle B Clinical and laboratory findings in subjects with hypercalcemia Acta Med Scand 200 355 1976
4 Christensson T Hellström K Wengle B Alveryd A & Wikland B Prevalence of hypercalcemia in a health screening in Stockholm Acta Med Scand 200 131 1976
5 Christiansen C Rodbro P & Lund M Incidence of anticonvulsant osteomalacia and effect of vitamin D controlled therapeutic trial Br Med J 4 695 1973
6 Dent C E Richens A Rowe D J F & Stamp T C B Osteomalacia with long term anticonvulsant therapy in epilepsy Br Med J 4 69 1970
7 Hahn T J Hendin B A Scharp C R Boisseau V C & Haddad J G Serum 25-hydroxycalciferol levels and bone mass in children on chronic anticonvulsant therapy N Engl J Med 292 550 1975
8 Hahn T J Hendin B A Scharp C R & Haddad J G Effect of chronic anticonvulsant therapy on serum 25-hydroxycalciferol levels in adults N Engl J Med 287 900 1972
9 Hunter J Maxwell J D Stewart D A Parsons V & Williams R. Altered calcium metabolism in epileptic children on anticonvulsants Br Med J 4 202 1971
10 Kraft D von Herrath D & Schaefer K Antikonvulsiva und Vitamin-D-Stoffwechsel Munch Med Wochenschr 116 1579 1974
11 Kruse R Osteopathien bei antiepileptischer Langzeittherapie Monatsschr Kinderheilkd 116 378 1968
12 Lidgren L & Walloe A Incidence of fractures in epileptics Acta Orthop Scand 48 356 1977
13 Lifshitz F & MacLaren N K Vitamin D-dependent rickets in institutionalized mentally retarded children receiving long term anticonvulsant therapy A survey of 288 patients J Pediatr 88 612 1973
14 Livingstone S Berman W & Pauli L L Anticonvulsant drugs and vitamin D metabolism. JAMA 224 1634 1973
15 Mosekilde L Christensen M S Hansen H H Melsen F & Norman A W Effect of 1 25-dihydroxycholecalciferol and 25-HCC on fractional intestinal calcium absorption in anticonvulsant osteomalacia Calcif Tissue Res (Suppl) 24 18 1977
16 Mosekilde L Christensen M S Lund B Sørensen O H & Melsen F The interrelationships between serum 25-hydroxycholecalciferol serum parathyroid hormone and bone changes in anticonvulsant osteomalacia Acta Endocrinol 84 559 1977
17 Mosekilde L & Melsen F Anticonvulsant osteomalacia determined by quantitative analysis of bone changes Acta Med Scand 199 349 1976
18 Murchison L E Bewsher P D Chesters M Gilbert J Catto G Law E McKay E & Ross H S Effects of anticonvulsants and inactivity on bone disease in epileptics Postgrad Med J 51 18 1975
19 Nordin B E C Metabolic bone and stone disease p 68 Churchill Livingstone Edinburgh 1973
20 — Calcium phosphate and magnesium metabolism pp 43–45 52 Churchill Livingstone Edinburgh 1976
21 Richens A & Rowe D J D Disturbance of calcium metabolism by anticonvulsant drugs Br Med J 4 73 1970
22 Sotaniemi E A Hakkarainen H K Puranen J K & Lahti R O Radiologic bone changes and hypocalcemia with anticonvulsant therapy in epilepsy Ann Intern Med 77 389 1972

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Fractional Intestinal Calcium Absorption in Epileptics on Anticonvulsant Therapy

Short Term Effect of 1,25 Dihydroxycholecalciferol and 25 Hydroxycholecalciferol

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ABSTRACT. Fractional intestinal ^{47}Ca calcium absorption (α) in 12 epileptic outpatients receiving chronic high dose anticonvulsant therapy was reduced ($p<0.05$) compared to 12 matched normal controls. Six of the epileptics were treated orally with 0.5 μg of 1,25 dihydroxycholecalciferol (1,25-DHCC) per day and six with 10 μg of 25 hydroxycholecalciferol (25-HCC) per day for 10 days. The α was determined before and after treatment and compared with the effect of 0.5 μg of 1,25 DHCC per day given for 10 days to 6 controls. An increase of the same order in α was found in all groups ($p<0.05$). No changes were observed in the serum levels of calcium, phosphorus, alkaline phosphatase or iPTH during treatment. Urinary calcium excretion was low in the epileptic patients and rose during treatment. The investigation demonstrates that the sensitivity of the intestine to 1,25 DHCC is normal in epileptic patients on anticonvulsant therapy and that 1,25-DHCC and 25-HCC in the given doses had an equal effect on the reduced intestinal calcium absorption.

Key words: intestinal calcium absorption, anticonvulsant osteomalacia, 1,25 dihydroxycholecalciferol, 25 hydroxycholecalciferol.

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Anticonvulsant drug induced osteomalacia (4, 11, 15) is considered to stem in part from induction of hepatic microsomal enzyme activity, resulting in decreased plasma ^{3}H cholecalciferol half life, increased turnover rate of 25-hydroxycholecalciferol (25-HCC) and more polar metabolites and reduced serum and tissue levels of active vitamin D metabolites (8, 13, 17). However, normal or increased serum levels of 1,25-dihydroxycholecalciferol (1,25 DHCC), the ultimate metabolically active vitamin D metabolite, have been reported in patients receiving anticonvulsant therapy (9), suggesting a hyporesponsiveness of intestine and bone to 1,25 DHCC.

The aim of the present investigation was to elucidate the influence of long term anticonvulsant therapy on fractional intestinal calcium absorption (α) in epileptic patients and to compare the sensitivity of the intestine to exogenous 1,25 DHCC in epileptic patients and in normal controls. Furthermore, the intestinal calcium absorption was related to the serum concentration of 25-HCC, and the effect of exogenous 25 HCC was investigated in epileptic patients.

SUBJECTS AND METHODS

The study comprised 12 epileptic patients, 6 women and 6 men aged 20–36 years (mean 27.4), receiving long term treatment with diphenylhydantoin in combination with 1 or 2 other anticonvulsants, and 12 healthy volunteers, 6 women and 6 men aged 21–35 years (mean 27.3). Six epileptics and 6 controls were treated with 0.5 μg of 1,25 DHCC per day orally for 10 days and 6 epileptics were treated with 10 μg of 25-HCC per day orally for 10 days. The anticonvulsant treatment was not changed in the treatment period.

The α was determined before and on the last day of treatment from the accumulation of ^{47}Ca in the forearm following an i.v. dose of 2 μCi ^{47}Ca in 2 ml saline containing 0.1 mg ^{40}Ca and later an oral dose of 20 μCi ^{47}Ca in 90 ml water containing 200 mg ^{40}Ca (6). The radioactivity of the forearm, measured after the oral dose, was cor-

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Abbreviations: 25 HCC=25-hydroxycholecalciferol, 1,25 DHCC=1,25-dihydroxycholecalciferol, α=fractional intestinal calcium absorption, PTH=parathyroid hormone, S-iPTH = serum immunoreactive PTH.

Table I *Chemical quantities in serum and urine in epileptic patients and in normal controls*

	S-calcium corrected (mmol/l)	S phosphorus (mmol/l)	S-alkaline phosphatase (U/l)	S-iPTH (pg/ml)	S-25-HCC (ng/ml)	U calcium (mmol/mol creatinine)
Epileptics (N=12)						
$\bar{x}$	2 398	1 18	172	76 3	18 4	236
S E	0 014	0 05	14	9 2	3 6	23
Controls (N=60)						
$\bar{x}$	2.521	1 18	147	67.5[a]	28 2[b]	430
S E	0 009	0 02	4	2 2	2 2	13
P	<0 01	n s	<0 02	n s	<0 02	<0 01

[a] N=76 [b] N=56

rected for residual radioactivity from the i v dose using the biological decay rate determined for each individual (6) The coefficient of variation of repeated measurements was 3 5% at the level of the normal mean (42%)

Serum 25-HCC was measured by a competitive protein binding assay (7) with a modification in the extraction procedure and the chromatographic step The coefficient of variation of repeated measurements at the level of 15 ng/ml was 13 5% The sensitivity in the routine assay was 0 8 ng/ml

Serum immunoreactive parathyroid hormone (S-iPTH) was measured on extracts of serum (3) The antibody used was antibovine PTH 211/32 (Burroughs Wellcome) and bovine PTH (1–84) was used for radioiodination The coefficient of variation was 16% for measurements within the normal range (30–105 pg/ml bovine equivalents MRC bPTH standard 71/324) and the sensitivity 10 pg bPTH present in the incubation mixture The assay mainmeasures native PTH (1–84)

Serum concentrations of calcium phosphorus and alkaline phosphatase were measured daily during the investigation period by standard laboratory methods Serum calcium was corrected for individual variations in serum protein concentration (14) The urinary 24-hour excretions of calcium and creatinine were determined daily on a nonrestricted diet The renal calcium excretion was expressed in mmol/mol creatinine excreted

Statistical significance of differences in group means was determined by Wilcoxon tests for two samples or for paired samples and correlation coefficients by Spearman s rank correlation (R)

RESULTS

Table I gives the biochemical values measured in the epileptic patients before treatment with vitamin D metabolites and in normal controls The individual values for serum calcium serum phosphorus serum alkaline phosphatase and urinary calcium excretion in the epileptics were calculated as the mean from 4 consecutive days The serum calcium and 25-HCC levels and the renal calcium excretion in the epileptics were reduced compared to controls The serum level of alkaline phosphatase in the epileptic patients was increased whereas no differences were found in serum concentrations of phosphorus and iPTH between the epileptics and the controls

The α was significantly reduced ($p<0$ 05) in the epileptic patients compared with sex and age matched normal controls (Fig 1) The α was unrelated to the serum levels of 25 HCC both in the epileptics ($R=0$ 18 $p>0$ 10) and in the controls ($R=$ 0 03 $p>0$ 10) A significant increase ($p<0$ 05) in α was found in the epileptics following treatment with both 25 HCC and 1 25 DHCC and in the controls following treatment with 1 25 DHCC (Fig 2) No significant differences were found between the groups in the absolute or relative increases in α during treatment Among all the individuals an inverse correlation was found between the increase in α and the pretreatment α value ($R=-0.53$ $p<0$ 05) (Fig 3) A significant increase ($p<0$ 05) in the renal calcium excretion was found in all three

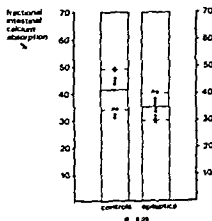


Fig 1 The α in epileptic patients receiving anticonvulsants and in normal controls (mean±S D)

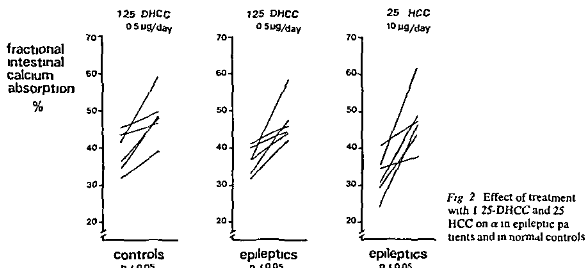


Fig 2 Effect of treatment with 1 25-DHCC and 25 HCC on α in epileptic patients and in normal controls

groups during treatment with vitamin D metabolites (Fig 4) The rise in the renal calcium excretion was prompt in the two groups treated with 1 25 DHCC in whom a nearly two-fold increase persisted from the third day of treatment In the epileptic patients treated with 25 HCC however the rise was slow a nearly two-fold increase being reached at the tenth day of treatment No differences were found between the groups in the maximum absolute or relative increase in renal calcium excretion during treatment or in the decrease after treatment The serum concentrations of calcium phosphorus alkaline phosphatase and iPTH were not affected by the treatment (Table II) The serum concentrations of 25-HCC were unchanged in those given 1 25 DHCC but significantly increased ($p<0\ 05$) in those given 25 HCC

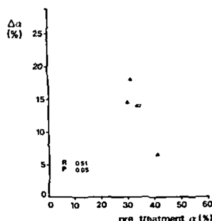


Fig 3 Relation between the increase in α (Δα) during treatment with vitamin D metabolites and the pretreatment α O=Controls receiving 1 25-DHCC ●=epileptics receiving 1 25-DHCC ▲=epileptics receiving 25-HCC

DISCUSSION

The present study confirms the findings in other reports on the biochemical changes in epileptic patients receiving chronic anticonvulsant therapy (1 10 11 15)

In the present patients, the α was reduced compared with sex and age matched normal controls This finding confirms the results of a previous study based on early blood radioactivity measurements

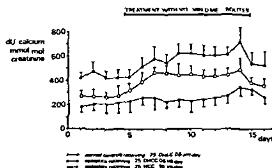


Fig 4 Urinary calcium excretion (mean±S E) in epileptic patients treated with 25-HCC ($n=6$) or 1 25-DHCC ($n=6$) and in controls treated with 1,25-DHCC ($n=6$)

Table II *Chemical quantities in serum and urine in epileptic patients and in controls before and on the last day of treatment with 0.5 μg 1,25-DHCC or 10 μg 25-HCC for 10 days*

		S-calcium corrected (mmol/l)	S-phosphorus (mmol/l)	S-alkaline phosphatase (U/l)	S-iPTH (pg/ml)	S-25-HCC (ng/ml)	U-calcium (mmol/mol creatinine)
Controls ($n=6$)							
Pretreatment	$\bar{x}$	2.49	1.16	131	63.4	34.7	431
	S.E.	0.01	0.04	9	16.1	1.4	49
Last day on 1,25-DHCC	$\bar{x}$	2.52	1.16	138	59.3	34.9	719
	S.E.	0.01	0.04	9	14.2	2.5	79
	p	n.s.	n.s.	n.s.	n.s.	n.s.	<0.05
Epileptics ($n=6$)							
Pretreatment	$\bar{x}$	2.41	1.19	183	73.2	24.5	261
	S.E.	0.01	0.04	22	13.1	6.3	33
Last day on 1,25-DHCC	$\bar{x}$	2.41	1.18	175	71.6	22.2	498
	S.E.	0.01	0.04	24	11.0	4.4	74
	p	n.s.	n.s.	n.s.	n.s.	n.s.	<0.05
Epileptics ($n=6$)							
Pretreatment	$\bar{x}$	2.39	1.17	161	79.4	12.2	212
	S.E.	0.01	0.04	21	14.4	2.7	51
Last day on 25-HCC	$\bar{x}$	2.41	1.18	174	72.3	19.7	3 6
	S.E.	0.01	0.04	25	13.8	1.6	55
	p	n.s.	n.s.	n.s.	n.s.	<0.05	<0.05

after oral ^{45}Ca in epileptic patients (16). Although reflecting the intestinal calcium absorption to some [extent] these results are influenced by the increased turnover in anticonvulsant osteomalacia (12) which will accelerate the disappearance rate of ^{45}Ca from the blood. This error is eliminated in the present study provided there is an identical distribution of the i.v. dose and the absorbed fraction of the oral dose.

The reduced intestinal calcium absorption in epileptic patients suggests a direct inhibition of the intestinal calcium absorption by anticonvulsant drugs and/or a decreased tissue level of metabolically active vitamin D metabolites. A direct inhibitory effect of diphenylhydantoin on the vitamin D-mediated calcium absorptive mechanism has been demonstrated in organ cultured embryonic chick duodenum (5). In the same study, duodenal calcium-binding protein (CaBP) and cAMP concentrations and ^{45}Ca uptake by the tissue declined in a dose-dependent fashion in the presence of diphenylhydantoin. However, a normal concentration of CaBP has been found in rat duodenal homogenates after pretreatment with diphenylhydantoin for 20 days in spite of a marked decrease in calcium absorption (2). Furthermore, normal or increased plasma levels of 1,25-DHCC have been reported in epileptic patients receiving anticonvulsant drugs suggesting a decreased sensitivity of the intestine to 1,25-DHCC (9).

The present study showed an equal increase in intestinal calcium absorption in the epileptic patients and in the controls following treatment with 1,25-DHCC indicating that the sensitivity of the intestine to exogenous 1,25-DHCC is normal in patients on anticonvulsant therapy. This supports the view that the decreased intestinal calcium absorption is caused mainly by a reduced tissue concentration of active vitamin D metabolites (8, 13, 17). Hence, at present the available results are conflicting and no single explanation exists for the reduced intestinal calcium absorption in epileptic patients.

The α was unrelated to the serum concentration of 25-HCC both in epileptics and in controls indicating that this metabolite is of minor importance for the intestinal calcium absorption. Exogenous 25-HCC, however, increased the intestinal calcium absorption in the epileptic patients. The sensitivity of the intestine to exogenous 25-HCC was about 20 times less than the sensitivity to 1,25-DHCC and the increase in urinary calcium excretion was slow compared with the increase following 1,25-DHCC.

This finding is in accordance with the accepted renal conversion of 25 HCC to more polar metabolites before action at target sites and with the finding of an impaired biological action of 25-HCC in rachitic chicks treated with phenobarbital (13) and in epileptic patients receiving chronic anticonvulsant therapy (12)

REFERENCES

1 Bouillon R Raynaert J Claes J H Lissens W & De Moor P The effect of anticonvulsant therapy on serum levels of 25-hydroxy vitamin D calcium and parathyroid hormone J Clin Endocrinol 41 1130 1975

2 Caspary W F Inhibition of intestinal calcium transport by diphenylhydantoin in rat duodenum Naunyn Schmiedebergs Arch Pharmacol *274 146 1972*

3 Christensen M S A sensitive radioimmunoassay of parathyroid hormone in human serum Employing a specific extraction procedure Scand J Clin Lab Invest 36 313 1976

4 Christiansen C Rødbro P & Lund M Incidence of anticonvulsant osteomalacia and effect of vitamin D Controlled therapeutical trial Br Med J 4 695 1973

5 Corradino R A Diphenylhydantoin Direct inhibition of the vitamin D_3 mediated calcium absorptive mechanism in organ cultured duodenum Biochem Pharmacol *25 863 1976*

6 Curtis F K Fellows H & Rich C Estimation of human calcium absorption by external radioisotope counting J Lab Clin Med 69 1036 1967

7 Haddad J G & Chyu K J Competitive protein binding radioassay for 25 hydroxycholecalciferol J Clin Endocrinol 33 992 1971

8 Hahn T J Birge S J & Scharp C R Phenobarbital induced alterations in vitamin D metabolism J Clin Invest 51 741 1972

9 Jubiz W Haussler M R McCain T A & Tolman K G Plasma 1,25-dihydroxyvitamin D levels in patients receiving anticonvulsant drugs *J* Clin Endocrinol 44 617 1977

10 Mosekilde L Christensen M S Lund B Sørensen O H & Melsen F The interrelationships between serum 25-hydroxycholecalciferol serum parathyroid hormone and bone changes in anticonvulsant osteomalacia Acta Endocrinol 84 559 1977

11 Mosekilde L. & Melsen F Anticonvulsant osteomalacia determined by quantitative analyses of bone changes Population study and possible risk factors Acta Med Scand 199 349 1976

12 Mosekilde L. Melsen F Christensen M S Lund B & Sørensen O H Effect of long term vitamin D_2 treatment on bone morphometry and biochemical values in anticonvulsant osteomalacia Acta Med Scand *201 303 1977*

13 Norman A W Bayless J D & Tsai H C Biologic effects of short term phenobarbital treatment on the response to vitamin D and its metabolites in the chick Biochem Pharmacol 25 163 1976

14 Pedersen K O Protein-bound calcium in human serum Quantitative examination of binding and its variables by a molecular binding model and clinical chemical implications for measurements of ionized calcium Scand J Clin Lab Invest 30 321 1972

15 Richens A & Rowe D J F Disturbance of calcium metabolism by anticonvulsant drugs Br Med J *4 73 1970*

16 Shafer R B & Nuttal F Q Calcium and folic acid absorption in patients taking anticonvulsant drugs J Clin Endocrinol 41 1125 1975

17 Silver J Neale G & Thompson G R Effect of phenobarbitone treatment on vitamin D metabolism in mammals Clin Sci Mol Med 46 433 1974

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Mexiletine in Treatment of Ventricular Arrhythmias

A Long Term Follow up

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ABSTRACT Mexiletine, a new drug effective in the treatment of ventricular arrhythmias, was given to 20 patients for approximately 2 years The study was designed to investigate the nature and prevalence of side-effects during long term therapy, and the degree of correlation between such effects and the serum level of the drug The methodology used to determine the serum level is described Mexiletine was well tolerated and serious side-effects were not seen In particular antinuclear factor was not detected during the treatment period The serum level of mexiletine was easily maintained within the therapeutic range, and most side-effects correlated closely with the drug level Mexiletine appears to be an effective alternative to currently available antiarrhythmic agents

Key words mexiletine ventricular arrhythmia.

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Mexiletine (Kö 1173 2-(2 6-dimethylphenoxy) 1 methylethylamine) is a new compound effective against ventricular arrhythmias Pharmacologically it resembles lidocaine but it is metabolised more slowly and can be given orally (Fig 1) Mexiletine belongs to class I b of antiarrhythmic drugs according to the classification of Vaughan Williams (13 18) The efficacy of available antiarrhythmic agents is often limited and side-effects are common For example procainamide may induce a systemic lupus erythematosus (SLE) like syndrome (6) quinidine can cause gastrointestinal side-effects (5) and β-blockers may unmask latent cardiac failure There are therefore good reasons to search for an effective antiarrhythmic drug which may be used on a long term basis with few side-effects We have administered mexiletine to 20 patients for about 2 years to find out if the drug fulfils these requirements

SUBJECTS AND METHODS

Twelve patients (group A) with ventricular arrhythmias of differing aetiology were given mexiletine for a mean period of 20 months (Table I) The initial dose was 200 mg three times daily but in some cases it was necessary to modify the dosage regime because of side-effects or lack of efficacy The patients were followed according to a strict protocol (Table II) Side-effects were registered and serum levels of the drug were measured The same doctor examined the patients at each visit Additional medication was given according to conventional indications

A further 8 patients (group B) were treated with mexiletine for 2–37 months (Table I) During the last 20 months the follow up was identical with that in group A except that different doctors saw the patients and 10-min ECGs were not always recorded at each examination

The effect of mexiletine compared with other antiarrhythmic drugs was studied more closely in 3 patients with ventricular arrhythmias after myocardial infarction We used an experimental design described by Johansson and Persson (8) in which quinidine disopyramide mexiletine and placebo were given for periods of 5 days consecutive treatment periods being separated by a 2-day wash-out interval. The ECG was recorded continuously by tape recorder and the number of ventricular ectopic beats was counted during the first 10 min of every hour

In addition the serum half life of mexiletine was studied in 5 patients with uncomplicated myocardial infarction and in 6 healthy volunteers The studies on the patients were conducted on the 3rd or 4th day after hospital admission Mexiletine was given orally in a dose of 400 mg after a 12 hour fast Serum levels were determined after 2 3 4 6 8 and 10 hours Furthermore we compared the half life of mexiletine at different times of the year (June Oct Jan) and also the diurnal variation in 2 healthy subjects

Determination of serum concentration of mexiletine

Mexiletine levels were determined by the following gas chromatographic method 1 ml serum was placed in a 15 ml glass-stoppered tube along with 2.5 µl 0 22 mg/ml [1] solution of 1-(2 6-dimethylphenoxy)2 aminopropan (inter

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Abbreviations SLE=systemic lupus erythematosus ANF=antinuclear factor

Table I. *Clinical data on the patients in groups A and B*

AMI=*acute myocardial infarction*, IHD=*ischaemic heart disease*, VES=*ventricular extra systoles*, VHD=*valvular heart disease*, AF=*atrial fibrillation*, MS=*myocardial sarcoidosis*

Pat. no	Sex	Year of birth	Diagnosis	Side-effects	Follow-up (mo)
Group A					
1	♂	14	AMI	Tremor	
2	♂	24	AMI	(Ataxia)	
3	♂	-07	IHD	Tremor	
4	♀	26	Idiopathic VES		
5	♂	25	AMI	Nausea	
6	♂	13	AMI		
7	♂	15	AMI		
8	♂	21	AMI	(Dysarthria) nausea	
9	♂	-08	IHD	Nausea, tremor	
10	♂	24	AMI	Nausea (positional vertigo)	
11	♂	12	AMI	Vertigo	
12	♂	18	AMI	Tremor vertigo nystagmus	
Group B					
13	♂	21	AMI		-0
14	♀	-02	VHD AF		7
15	♂	26	AMI		8
16	♀	-04	MS		33
17	♂	22	AMI		9
18	♂	12	AMI		2
19	♂	-05	IHD	Nausea	13
20	♂	18	AMI		6

nal standard), 15 μl 2 M sodium hydroxide and 3 ml benzene. The tube was mechanically shaken for 10 min. After centrifugation, the benzene layer was transferred to a fresh tube and dried by addition of anhydrous sodium sulphate. A 2 ml aliquot of the benzene phase was transferred to a conical tube and 20 μl heptafluoro-butyric anhydride (Pierce) was added. After incubation at 50°C for 15 min, the solvent and excess reagent were removed by a stream of dry air. The residue, containing the fluoroacyl derivative of mexiletine, was dissolved in 1 ml benzene and 2 μl of this was injected into the gas chromatograph.

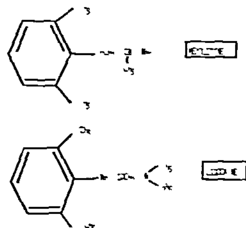


Fig. 1. Structural formulas of mexiletine and lidocaine.

A Varian 2100 gas chromatograph, fitted with a scandium tritide electron capture detector, was used. The column was 1.85 m×2 mm (i.d.) glass, packed with 2.5% XE 60 on Chromosorb G-PH 80/100 mesh. The operating conditions were: column oven temperature 170°C, detector temperature 260°C and injection port temperature 500°C. The carrier gas was nitrogen, and the flow rate was 31.5 ml min^{-1}.

Quantitation was achieved from a standard curve prepared for each batch of samples, using known amounts of mexiletine and internal standard added to pooled human serum and carried through the entire process. The ratios of mexiletine peaks to those of the internal standard were plotted against mexiletine concentrations and gave a straight line passing through the origin. Duplicate analyses of sera, containing added mexiletine in the concentration range 1–20 μmole l^{-1}, gave a standard deviation of 4.5%. Serum concentration values are given in SI units. To obtain the values in μg ml^{-1} the figures should be divided by 5.6.

A sample of 2-(2,6-dimethylphenoxy)-1-methylethylamine (Kö 1173)—used as internal standard in our analyses—was supplied by C. H. Boehringer Sohn, Ingelheim/Rhein, West Germany.

RESULTS

Most patients achieved serum levels of 2.8–8.4 μmol/l^{-1}, which we found to be the therapeutic range. In some patients, the value periodically fell

Table II *Protocol for the follow up in group A*

BP=blood pressure Hb=haemoglobin concentration RBC=red blood cell count L=leucocytes ASAT=aspartate aminotransferase ALAT=alanine aminotransferase ALP=alkaline phosphatase GT=glutamyl transpeptidase LD lactate dehydrogenase ANF=antinuclear factor

Time	Tests
Before treatment	BP pulse rate 10-min ECG Hb RBC L trombocytes folate cobolamines bilirubin ASAT ALAT ALP GT LD Na K creatinine urate blood glucose cholesterol triglyceride ANF
3 days	BP pulse rate 10-min ECG mexiletine/s
1 month	Same as at 3 days
3 months	Same as at 3 days + laboratory test before treatment
6 months	Same as at 3 months
12 months	Same as at 3 months
18 months	Same as at 3 months

just below the lower limit but the fall was due to temporary reduction of dosage One patient (no 12) had a serum level of 11 2 μmol/l ¹ while receiving 1 200 mg mexiletine daily

No drug related laboratory abnormalities were seen during the course of the study One patient (no 5) had two transient rises in liver enzyme values but they returned to normal in spite of continuation of mexiletine therapy No rise was recorded in the titre of antinuclear factor (ANF) In summary no toxic effects were noted on hepatic renal or haemopoietic tissue

CASE REPORT

Case 16 female born in 1904 Sarcoidosis diagnosed in 1969 on the basis of skin biopsy Ventricular ectopic beats noted in 1970 presumed to be due to myocardial sarcoidosis An episode of ventricular tachycardia in 1970 was treated with corticosteroids and procainamide but the patient developed an SLE syndrome with high ANF values The introduction of quinidine resulted in a drug fever Mexiletine was given continuously following a further episode of ventricular tachycardia in 1974 Subsequently the ANF disappeared from serum and the patient remained free from further episodes of serious ventricular arrhythmia until her death in Aug 1977 from myocardial failure Autopsy revealed heavy myocardial fibrosis containing epithelioid granulomas

Subjective side-effects that were noted included a fast tremor of the fingers in 4 patients vertigo in 2 ataxia in 1 and nausea in 4 Tremor and nausea were seen almost exclusively in the first 3–4 weeks of treatment and disappeared thereafter without reduction of dosage One patient (no 12) was subject to tremor for a longer time and in his case the symptom was correlated with a high serum value of the drug (11 2 μmol/l ¹) The remaining CNS side effects also correlated well with the drug level in serum Side effects were more common at the upper limit of the therapeutic serum level

One patient (no 10) had two attacks of benign positional vertigo but in spite of intensive otovestibular investigation before and after mexiletine we have not been able to attribute this symptom to a medical side effect

Three patients died during the study for reasons which could not be related to inadequate antiarrhythmic treatment Two (nos 12 and 16) died from myocardial failure after 6 and 36 months of treatment and one (no 11) following pulmonary embolism after 4 months

The results of the three long term ECGs recorded on tape are seen in Table III In these patients the effects of mexiletine seem to be similar to those of the other drugs

In the five patients with myocardial infarction the mean serum half life was 8 2 hours and 10 0 hours in the six healthy volunteers No seasonal or diurnal variation in the elimination pharmacokinetics was found in two healthy subjects studied

DISCUSSION

Since available antiarrhythmic drugs often give rise to side effects during long term treatment there is a need for an alternative drug According to previous reports mexiletine seems promising in this respect (3 7 16 17) It is almost completely absorbed from the gastrointestinal tract resulting in a stable serum concentration within the therapeutic range when a standard dose is given Two patients who were

Table III *Mean rate of ventricular extra systoles/min in three patients during five-day periods on various antiarrhythmic drugs and placebo*

Pat no	Quinidine	Placebo	Disopyramide	Mexiletine
21	0 24	0 31	0 17	0 25
22	0 31	5 4	1 0	0 15
23	4 3	4 1	1 8	3 9

given a high dose of 1 000 and 1 200 mg daily experienced side effects Factors which influence absorption are severe cardiac failure and the concomitant administration of narcotic analgesics (7) The drug is metabolised in the liver to several metabolites primarily by oxidation and reduction (1) Biological activity therapeutic or toxic has not been established for any of the metabolites In view of the metabolic processes involved it is possible that the rate of metabolism may be influenced by smoking or exposure to enzyme inducing agents such as phenobarbitone The percentage of unchanged drug in the urine is pH dependent At normal urinary pH amounts of 10–23 % of ingested doses are recovered unchanged but this value rises to almost 50% at pH 5 0 and falls to almost negligible amounts at pH 8 0 (9 10)

There are no reports to our knowledge of interactions between mexiletine and other important drugs used in the treatment of cardiac disorders with the exception of narcotic analgesics In our experience no interaction was noted when mexiletine was given to 4 patients who had been previously stabilised on dicoumarol therapy

Mexiletine has no influence on sinus rate or atrial and AV node refractoriness The conduction time in the AV node is unchanged or prolonged just as the HV interval The effective refractory period in e AV node is usually unchanged while the func onal refractory period is usually prolonged Both the relative and the effective refractory periods of the His Purkinje system are increased (13 14) Thus the drug belongs to group Ib of the Vaughan Williams classification Roos et al (14) claim that the drug should not be given to patients with conduction defects in or distal to the AV node or to subjects with known sinus node dysfunction In the present study we did not include patients with these conduction defects

Prescott et al (12) found a plasma half life of 9 7±1 9 h in healthy subjects and 12 1±4 0 h in patients while Talboy et al (16) noted values of 10 2±0 8 h and 18 7±1 8 h respectively We have not been able to confirm this difference perhaps because our patients had minimal circulatory disturbances

The purpose of our studies has not been to evaluate the efficacy of mexiletine This has already been shown to be comparable with that of other antiarrhythmic agents (3 7 16 17) On the basis of our different studies however the drug seems to be effective We have no reason to believe that any of the 3 deaths in our series was due to inadequate antiarrhythmic control

The side effects can be classified into 2 groups The first group consists of gastrointestinal symptoms, mainly nausea and these effects seem to be related to the size of the single dose They appear most often at the beginning of treatment and tend to disappear in a few weeks One can in most cases diminish the discomfort by giving several smaller doses instead of 3 standard doses The second group comprises the CNS side effects These are related to the total daily dose and through it to the serum concentration Again in this group certain side effects mainly tremor, appear during the first weeks of treatment and then disappear without reduction of dosage Therefore when dealing with side effects of this kind, one should not discontinue therapy, since a slight reduction of dosage is sufficient in most cases In one patient (no 8) we have after dosage reduction added propranolol with good therapeutic effect

A rise in ANF has not been seen Induction of SLE has not been reported in the literature It is of considerable interest that ANF can be normalised in patients with procainamide induced SLE after changing to mexiletine (4 17) One of our patients (no 16) illustrates this phenomenon

Thus the tolerability seems good and we have not been forced to discontinued mexiletine administration because of side effects in any case In uncomplicated long term treatment with mexiletine there is hardly any indication for routine determination of the serum level However when the therapeutic effect is difficult to evaluate and when there is a discrepancy between the expected and the observed effect determination of the serum concentration is indicated

It is not known whether long term treatment with mexiletine has a cardiodepressive effect Short term studies regarding haemodynamic conditions (11 15) show a cardiodepressive effect comparable with lidocaine or no such effect at all One may speculate as to whether mexiletine contributed to cardiac failure in 2 of the 3 deaths among our patients In the first case we had a patient (no 12) with chronic glomerulonephritis and uraemia who had had 4 myocardial infarctions and in the seond the patient (no 16) had myocardial sarcoidosis, both conditions that could very well explain the cardiac failure Only haemodynamic studies before and af

ter long term treatment can throw light on this problem

It is concluded that mexiletine can be given for a long time as a safe alternative to other anti arrhythmic drugs and is usually well tolerated Serious side-effects are very rare The drug is specially valuable when one wishes to avoid the gastrointestinal effects of quinidine or the SLE inducing effects of procainamide While mexiletine may be given intravenously it should not be regarded as a first line drug for this route of administration However it can be of value in lidocaine resistant arrhythmias and in digitalis toxicity (2 16)

REFERENCES

1 Beckett A H & Chidomere E C The distribution metabolism and excretion of mexiletine in man Postgrad Med J (Suppl) 1 60 1977
2 Campbell N P S Chaturvedi N C Shanks R G Kelly J G Strong J E & Adgey A A J The development of mexiletine in the management of ventricular dysrhythmias Postgard Med J (Suppl) 1 114 1977
3 Campbell R W F Talbot R G Dolder M A Murray A Prescott L E & Julian D G Comparison of procainamide and mexiletine in prevention of ventricular arrhythmias after myocardial infarction Lancet 1 1257 1975
4 Campbell R W F Talbot R G Julian D G & Prescott L E Long term treatment of ventricular arrhythmias with oral mexiletine Postgrad Med J (Suppl) 1 146 1977
5 Eriksson J E Hanson A Horlin R Johansson B W Ohlsson O Otto U & Syren G Evaluation of Quinidine Lipettes®—a sustained released preparation Acta Med Scand 205 53 1979
6 Henningsen N C Cederberg Å Hanson A & Johansson B W Effects of long term treatment with procainamide Acta Med Scand 198 475 1975
7 Jewitt D McComish M & Jackson G Ambulatory monitoring in the controlled assessment of anti arrhythmic drug therapy Postgrad Med J (Suppl) 7 67 1976
8 Johansson B W & Persson S Disopyramide in patients with ventricular premature beats An experimental model and preliminary results J Int Med Res (Suppl) 1 96 1976
9 Kiddie M A & Kaye C M The renal excretion of mexiletine (Ko 1173) under controlled conditions of urine pH Br J Clin Pharmacol 1 86 1974
10 Kiddie M A Kaye C M Turner P & Shaw T R D The influence of urinary pH on the elimination of maxiletine Br J Clin Pharmacol 1 229 1974
11 Pozenel H Klinisch-haemodynamische Untersuchungen mit Mexiletine—einem neuen Antiarrhythmikum Herz/Kreisl 9 148 1977
12 Prescott L F Pottage A & Clements J A Absorption distribution and elimination of mexiletine Postgard Med J (Suppl) 1 50 1977
13 Robinson C McComish M Crook B Kitson D & Jewitt D Clinical electrophysiological effects of mexiletine a new local anaesthetic type of antiarrhythmic drug In 7th Eur Congr Cardiol Amsterdam Abstract book I p 360 June 20–25 1976
14 Roos J C Paalman A C A & Dunning A J Electrophysiological effects of mexiletine in man Br Heart J 38 1.62 1976
15 Shaw T R D The effect of mexiletine on ventricular ejection A comparison with lignocain and propranolol Postgrad Med J (Suppl) 1 69 1977
16 Talbot R G Clark R A Nimmo J Neilson J M M Julian D G & Prescott L F Treatment of ventricular arrhythmias with mexiletine (Ko 1173) Lancet 2 399 1973
17 Talbot R G Julian D G & Prescott L F Long term treatment of ventricular arrhythmias with oral mexiletine Am Heart J 91 vol 1 58 1976
18 Vaughan Williams E M Mexiletine in isolated tissue models Postgrad Med J (Suppl) 1 30 1977

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Kinetics of Hemodynamic and Electrocardiographic Changes Following Intravenous Disopyramide

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ABSTRACT Changes in hemodynamic and ECG variables following disopyramide (1 7 mg=3 9 mmol/kg b wt) were studied in 9 patients with ventricular arrhythmias All patients displayed a marked reduction in the number of ventricular ectopic beats, all except one exhibiting complete abolition of the arrhythmia for at least 30 min QT and QRS intervals showed statistically significant prolongations, and thereafter decreased exponentially with time Above a certain concentration threshold that varied between the patients, systolic time intervals and aortic $(dp/dt)_{max}$ showed linear changes with increasing drug serum levels Changes in diastolic pulmonary artery pressure showed no simple relationship with disopyramide concentration or time after injection In 3 out of 4 patients studied, there was a good correlation between the lowest level of disopyramide that elicited both an antiarrhythmic effect and a demonstrable decrease in cardiac contractility

Key words disopyramide cardiodepressive drugs hemodynamics serum concentration systolic time intervals ventricular arrhythmia
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Disopyramide (Durbis®) is an antiarrhythmic drug that effectively suppresses ventricular ectopic activity after i v or oral administration (7 8 15 21) Electrophysiological studies in man have shown a prolongation of the refractory period of the left ventricle (19) and an increase in the His–Purkinje conduction time (12) The effect on the resting ECG seems to vary there is little effect on the normal heart (14) whereas in heart disease a prolongation of ECG time intervals may occur after i v or oral administration (7 8 15) The drug has a negative inotropic effect which may be of clinical significance (8 10)

The pharmacokinetics of the drug have been studied in normal volunteers (5) Absorption after oral medication is rapid and about 80% of the administered dose is taken up The first pass elimination is low (10%) and systemic elimination occurs mainly by glomerular filtration of unchanged drug In cardiac patients the elimination phase half life has been shown to vary between 10 and 22 hours (7) which is considerably longer than reported for healthy volunteers (about 7 h) (5) Lowest plasma concentration suppressing ventricular ectopic beats (VEBs) varied between 1 4 and 7 0 μg/ml in one study (9)

In the present study hemodynamic ECG and antiarrhythmic effects of i v disopyramide have been correlated with the serum concentration of the drug

PATIENTS AND METHODS

Nine patients with persistent ventricular arrhythmias were studied The patients and the experimental procedure have been presented in a previous study (8) Individual characteristics and basal hemodynamic findings are given in Table I In all patients except one (no 5) catheters were introduced into the aorta superior vena cava and pulmonary artery Disopyramide phosphate 1 7 mg=3 9 mmol/kg b wt was administered i v over 2 min BP and ECG were continuously monitored and recorded on magnetic tape for subsequent off line analyses (16) Cardiac output was measured by the dye dilution technique A selection was made of variables showing statistically significant changes within 10 min following disopyramide administration measurements were made at −5 0 5 10 20 30 60 120 and 180 min The variables included diastolic pulmonary artery pressure (DPAP) pre ejection period (PEP) PEP divided by left ventricular ejection time (LVET) maximal first derivative of the aortic pressure curve ($Ao(dp/dt)_{max}$) and the QRS and QT

Abbreviations VEBs=ventricular ectopic beats DPAP=diastolic pulmonary artery pressure PEP=pre ejection period LVET=left ventricular ejection period $Ao(dp/dt)_{max}$=maximal first derivative of the aortic pressure curve QT =QT interval corrected for heart rate

Table I *Some data on and basal hemodynamic findings in the patients studied*
S=systolic D=diastolic MI=myocardial infarction

Pat no	Sex	Age (y)	BSA (m)	Heart rate (min⁻¹)	Pressures (mmHg) Aorta S	D	Pulm artery S	D	Cardiac output (l×min⁻¹)	Diagnoses besides arrhythmias
1	♀	75	1 61	93	144	67	20	8	3 1	Hypertension
2	♂	54	1 80	92	100	73	31	11	5 0	Acute MI
3	♂	67	1 78	115	147	85	53	28	5 8	Previous MI+diabetes
4	♀	67	1 67	68	140	71	34	14	4 3	Coronary arteriosclerosis
5	♀	34	1 77							Primary cardiomyopathy
6	♂	65	2 11	80	142	87	48	24	4 0	Previous MI+angina pectoris
7	♂	64	1 99	107	133	80	20	8	6 2	Previous MI+angina pectoris
8	♀	66	1 59	53	168	77	20	9	3 3	Angina pectoris
9	♂	67	1 78	79	90	64	40	16	3 2	Previous MI

intervals The latter variable was corrected for heart rate by Bazett s method (1) DPAP was taken as a measure of left ventricular end-diastolic pressure assuming a normal pulmonary vascular resistance in all patients Changes in systolic time intervals (PEP and PEP/LVET) and $Ao(dp/dt)_{max}$ were regarded as indices of changes in cardiac contractility (14 17) LVET was corrected for heart rate (22)

A bipolar ECG at a paper speed of 10 mm/sec was recorded continuously from 6–72 min before to 180–300 min after the administration of disopyramide VEBs were diagnosed from generally accepted criteria and counted each minute The evaluation of the antiarrhythmic effect was based on the hypothesis of a normal distribution of the number of VEBs before disopyramide administration (7) and on the calculation of a 95% confidence interval of VEB frequency per 6 min during the control period (24–72 min) An antiarrhythmic effect was considered to be present as long as the VEB frequency following disopyramide administration remained below the lower limit of this confidence interval (7) Even though the VEB frequency may not have been normally distributed there are reasons to believe that this would not invalidate the present method for assessing the antiarrhythmic effect in individual patients The concentration of disopyramide at the end of the antiarrhythmic period was obtained by linear interpolation In patient 1 it was impossible to estimate the confidence interval due to a too short control period and in patients 4 and 9 it included zero i e no or very few VEBs were counted during one or more 6-min periods prior to drug administration These patients were therefore excluded from the analysis of the antiarrhythmic effects of disopyramide

Blood samples (10 ml) were drawn into vacutainers 0 10 30 60 120 180 360 and 720 min after disopyramide administration The zero time specimen was used as a serum blank Serum was separated after clotting and stored frozen Disopyramide assay was carried out by a spectrofluorimetric method slightly modified from Ranney et al (18) The samples were processed in duplicate The method for disopyramide assay was evaluated by analyzing serum to which a known amount of drug had been added deliberately Assays were performed 4 times at the following concentrations 0 00 0 32 0 64 1 76, 2 51 3 77 and 7 53 µg/ml The accuracy of the method was defined as the difference between these concentration values and the mean value of each sample The precision of the method was expressed as the relative S D at each concentration level The method was also evaluated by comparing results from a spectrofluorimetric method with results from a gas chromatographic method slightly modified from Hutsell and Stachelski (9) For this comparison disopyramide was added to human plasma in vitro and analyzed by both methods

The relationships between disopyramide concentration and changes in contractility indices and DPAP were described by linear equations The concentration at which an increase of 10% in PEP occurred was obtained graphically and arbitrarily taken as a threshold value above which a reduction of cardiac contractility occurred Regression analyses were performed by the least squares method

RESULTS

Evaluation of the methods for disopyramide assay

The results are presented in Table II The accuracy and precision of the method were acceptable for serum levels above 0 6 µg/ml (2 6 µmol/l) There was a satisfactory correlation (r=0 99) between the spectrofluorimetric and the gas chromatographic method as shown in Fig 1

Serum concentrations in individual patients

Serum concentration curves from all patients are shown in Fig 2 Ten minutes after the start of the injection when the first sample was taken individual serum levels ranged from 2 0 to 8 4 µg/ml (mean 5 2) Six hours after the injection they varied

Table II *Spectrofluorimetric determination of disopyramide added to human serum in vitro*

S D calculated from $\sqrt{\Sigma d^2/2n}$ where d=difference between added and analytical concentration n=4

Added conc (μg/ml)	Mean analytical conc (μg/ml)	S D (μg/ml)	Relative S D (%)
0 00	0 00	0 00	-
0 32	0 25	0 06	19
0 64	0 64	0 03	5
1 26	1 24	0 04	3
2 51	2 68	0 16	6
3 77	3 70	0 07	2
7 53	7 31	0 18	3

between 0 5 and 3 3 μg/ml The decline of log disopyramide against time was linear in six patients with a correlation coefficient above 0 99 for sample points at 2–12 hours after the injection In the other three patients correlation varied between 0 84 and 0 96

Effect of disopyramide on the number of VEBs

In six patients the number of VEBs per 6 min during the control period was significantly above zero the average number before disopyramide was 94 decreasing to 5 during the period of antiarrhythmic effect (Table III) Antiarrhythmic effect of disopyramide lasted for 36–>200 min and was still present in patients 3 and 8 at the end of the monitoring period so the total length of the antiarrhythmic period could not be determined The lowest effective antiarrhythmic concentration ranged from 2 5 to 5 5 μg/ml

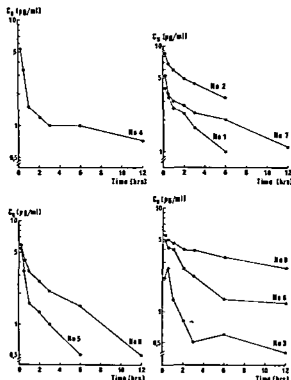


Fig 2 Log disopyramide concentration in relation to time following i v disopyramide (1 7 mg/kg b wt) in 9 patients

Changes in QRS and QT intervals

Disopyramide prolonged the QRS and QT intervals QT time could be determined at all measuring points in 6 patients The average QT interval cor

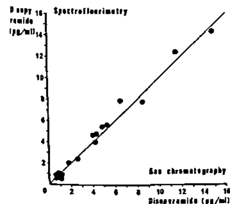


Fig 1 Correlation between the spectrofluorimetric and the gas chromatographic method used to determine disopyramide added to plasma in vitro

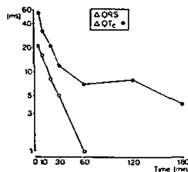


Fig 3 Relationship between mean prolongation of some time intervals in the ECG and time after i v disopyramide in 6 patients

Table III *Antiarrhythmic effects of i v disopyramide (1 7 mg/kg b wt)*

Pat no	No of VEBs per 6 min before disopyramide (mean±S D)	Duration of control period (min)	Average no of VEBs per 6 min during period of antiarrhythmic effect	Time (min after administration) when VEB suppression ended	Serum level of disopyramide (µg/ml)	
					when VEB suppression ended	when 10% PEP increase occurred
1	121	6				2 4
2	127± 8	24	5	84	5.5	5 7
3	85± 8	48	(1)	180	1 0	–
4	10± 7	48				2 5
5	196±13	54	11	42	2 6	
6	31±10	72	1	36	4 1	4 2
7	23± 8	60	1	160	2 4	5 2
8	116± 7	54	(26)	200	2 0	2 0
9	51±25	66				4 6

rected for heart rate (QT_c), and the average QRS interval in these patients are shown in Table IV After reaching peak values the QRS intervals de creased monoexponentially with time the shorten ing of QT time displaying a more complex pattern (Fig 3) with an early rapid fall partly reflecting the rapid change in the average QRS interval and a slower component

Change in DPAP

The increase in group mean DPAP values after disopyramide administration did not change with time in any simple mathematical way

Relationship between serum disopyramide and changes in PEP PEP/LVET $Ao(dp/dt)_{max}$ and DPAP

In most patients individual values for PEP prolongation and drug concentration were linearly related above an approximate concentration threshold (Fig 4) Below this threshold there was apparently only a minimal effect on contractility as expressed by changes in PEP The concentration at which a 10% increase of PEP occurred is presented in Table III It was not possible to es timate the threshold value in patient 3 and in patient 4 no increase in PEP was seen despite a maximal concentration of 5 2 µg/ml The lowest effective antiarrhythmic concentration and the low est concentration at which a negative hemodynamic effect of disopyramide could be detected were about the same in 3 out of the 4 patients in whom this relationship could be studied (Table III)

The group means of linear correlation coeffi cients for the relationship between disopyramide concentration and changes in PEP PEP/LVET $Ao(dp/dt)_{max}$ and DPAP in individual patients were

Table IV *Average QRS and QT intervals (mean±S D) following i v disopyramide in six pa tients*

Time after injection (min)	QRS (msec)	QT (msec)
−5	85±22	337±23
5	106±19	392±39
10	101±20	369±36
20	93±20	358±30
30	90±18	349±32
60	86±17	345±23
120	84±23	345±23
180	82±20	341±20

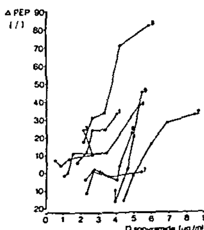


Fig 4 Relationship between increase in PEP and diso pyramide concentration in 8 patients

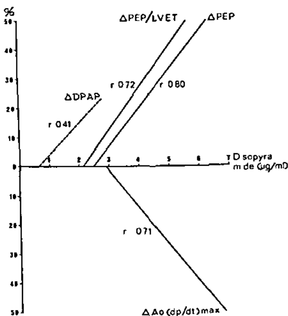


Fig 5 Averaged equations from 8 patients showing relationship between disopyramide concentration and changes in indices of cardiac contractility and DPAP

0 80 0 72 −0 71 and 0 41 respectively Thus the degree of linearity between changes in disopyramide concentration and the contractility indices was relatively strong whereas the corresponding relationship regarding DPAP was weak Fig 5 shows the average slope coefficients and intercepts for the various contractility indices vs drug concentration The indices of contractility showed similar relative changes for a given increase in disopyramide concentration However the lowest values above which the group means indicated a change varied between the indices

DISCUSSION

This study on the effects of disopyramide includes patients with imminent cardiac failure (Table I) Antiarrhythmic treatment per se was thought to be essential to improve the condition of these patients In the others antiarrhythmic treatment was indicated by significant cardiac symptoms A transient further increase of 5–10 mmHg was observed in patients with the highest pulmonary artery pressures This rise was not accompanied by any subjective discomfort

There was a marked reduction of the number of VEBs in each of the patients studied all except one exhibiting complete abolition of the arrhythmia for at least 30 min This antiarrhythmic effect of the dose given 1 7 mg/kg b wt i v over 2 min corresponds well with the results of earlier reports (15)

The individual time courses of the serum concentration (Fig 2) varied considerably Most of the subjects showed a markedly longer half life (elimination phase) than that reported for healthy male individuals by Ranney et al (18) In both studies a spectrofluorimetric method was used for disopyramide assay However as the results include possible metabolites of the drug the calculation of the half life for disopyramide did not seem to be warranted in the present study Furthermore the blood sampling period was not long enough to establish a pharmacokinetic model with acceptable accuracy On the other hand the method used here for disopyramide determination has a slightly higher accuracy than the specific gas chromatographic method (7) It has been shown that the dominant metabolite of disopyramide possesses a negative hemodynamic effect although less marked than that observed at a similar concentration of unchanged drug (14) From this point of view it may be preferable to correlate the hemodynamic effects of disopyramide with concentration values obtained by the less specific fluorimetric method It has been shown however that only small amounts of the dominant metabolite are present in plasma within the first hours following i v administration (5)

In the present study the negative inotropic effect of disopyramide was linearly related to the drug concentration above a certain threshold (Figs 4 and 5) However this relationship was tested only within a fairly narrow concentration range and cannot have been more than minimally influenced by concentration-dependent variations in protein binding (3) In contrast to findings regarding most other tested drugs disopyramide did not show a linear correlation between measured effects and log concentration

A transient negative inotropic effect following i v disopyramide in doses somewhat higher than those used by us has been reported (10) Our findings indicate that even the lowest dose resulting in an antiarrhythmic effect may be associated with signs of myocardial depression (10% increase in PEP Table III) Increases in PEP and QRS time intervals in individual patients are compared in Fig 6 Patient 5 in whom catheterization faile

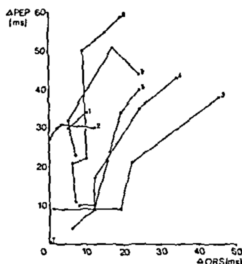


Fig 6 Relationship between prolongations of QRS interval and PEP in 8 patients

not be included in the graph As shown in Fig 6 most patients exhibited a more marked increase in PEP than in QRS intervals Intraventricular conduction defects can be expected to prolong PEP No conclusions can be drawn however concerning the extent to which the observed prolongation of PEP was caused by a delay in intraventricular conduction or by a depression of myocardial contractility

A statistically significant increase in the systolic ime intervals and $Ao(dp/dt)_{max}$ persisted 60 min after drug administration A statistically significant increase in DPAP was present for only 5–20 min Systolic time intervals and $Ao(dp/dt)_{max}$ were probably more sensitive measures of the cardiac effects of disopyramide than DPAP the latter variable supposedly being more influenced by hemodynamic adjustments induced by autoregulatory mechanisms Niarchos (15) who used a specific chromatographic method for disopyramide assay observed prolongations of the PR and QT intervals and a fall of diastolic BP with serum levels exceeding 3 6 µg/ml in patients without severe myocardial disease who were given disopyramide orally He concluded that the drug was safe below this concentration level in the patient category studied The present study included patients with severe myocardial disease and 3 of 8 cases studied revealed signs of negative inotropic effect at serum levels below the above concentration (Fig 4) It should be pointed out that the side effects associated with a given brief peak concentration following a single i v dose may be less marked than those occurring at the same but more prolonged concentration level following oral administration

The observed prolongation of the QT time is in accordance with the findings of Niarchos (15) and Hinderling and Garrett (4) The latter authors reported a peak of the RT interval at 0–2 min after disopyramide 2 mg/kg i v in healthy volunteers and a monoexponential decay with an apparent half life of 20 min The present findings are consistent with a biexponential decay of the QT interval within the first hour following drug administration, one rapid component comprising the decrease in the QRS interval within the first 25 min to about 1/5 of the initial value of the QT prolongation and one slower resulting from the abbreviation of the repolarization phase (Fig 3) It is conceivable that this course of the decay of the QT interval reflects a difference in the rate of drug loss from receptors responsible for the prolongation of the action potential due to decreased rate of phase 0 of the depolarization (2 23) and to increased refractory periods (20) respectively

In summary, the present findings indicate a close relationship between the antiarrhythmic and negative inotropic effects of disopyramide A prolongation of PEP (or PEP/LVET) which can be measured by non invasive techniques appeared to be a more sensitive measure of reduced cardiac contractility than a change in DPAP On the other hand the largest changes in PEP were seen in patients with high initial DPAP values (8) PEP showed a linear relationship to disopyramide concentration All patients except one showed a prolongation of the QRS interval with some tendency for these changes to parallel those in PEP The observations bear out the importance of a slow injection rate of the drug with constant surveillance of the patient s clinical condition especially if imminent or overt heart failure is present

REFERENCES

1 Bazett H C An analysis of the time relationship in the electrocardiogram Heart 7 353 1920
2 Danilo P & Rosen M Cardiac effects of disopyramide Am Heart J 92 532 1976
3 Hinderling P H Bres J & Garrett E R Protein binding and erythrocyte partitioning of disopyramide and its monodealkylated metabolite J Pharm Sci 63 1684 1974

4 Hinderling P H & Garrett E R Pharmacodynamics of the antiarrhythmic disopyramide in healthy humans Correlation of the kinetics of the drug and its effects J Pharmacokin Biopharm 4 231 1976

5 — Pharmacokinetics of the antiarrhythmic disopyramide in healthy humans J Pharmacokin Biopharm 4 199 1976

6 Heissenbuttel R H & Bigger J T Jr The effect of oral quinidine on intraventricular conduction in man. Correlation of plasma quinidine with changes in QRS duration Am Heart J 80 453 1970

7 *Hulting J & Jansson B Antiarrhythmic and el*ectrocardiographic effects of single oral doses of disopyramide Eur J Clin Pharmacol 11 91 1977

8 Hulting J & Rosenhamer G Hemodynamic and electrocardiographic effects of disopyramide in patients with ventricular arrhythmia Acta Med Scand 199 41 1976

9 Hutsell T C & Stachelski S J Determination of disopyramide and its mon N-dealkylated metabolite in blood serum and urine J Chromatogr 106 151 1975

10 Jensen G Sigurd B & Uhrenholt A Hemodynamic effects of intravenous disopyramide in heart failure Eur J Clin Pharmacol 8 167 1975

11 Karim A The pharmacokinetics of Norpace Angiology (Suppl) 1 part 2 1975

12 Marrott P K Ruttley M S T Winterbottam J T & Muir J R. A study of the acute electrophysiological and cardiovascular action of disopyramide in man Eur J Cardiol 4 303 1976

13 Marshall R The pharmacology of disopyramide (discussion) J Int Med Res (Suppl) 1 13 1976

14 Martin E Shaver J Thompson M Reddy S & Leonard J Direct correlation of external systolic time intervals with internal indices of left ventricular function in man Circulation 44 419 1971

15 Niarchos A P Disopyramide Serumlevel and arrhythmia conversion Am Heart J 92 57 1976

16 Nygårds M E. Tranesjo J Atterhog J H Blomqvist P Ekelund L-G & Wigertz O On line computer processing of pressure data from cardiac catheterizations Comp Progr Biomed 5 272 1976

17 Piriano L Wilson R & Traber D Use of the aortic pulse as an index of myocardial contractile force Tex Rep Biol Med 28 87 1970

18 *Ranney R. E Dean R R Karim A & Rad*zialowski F M Disopyramide phosphate pharmacokinetic relationships of a new antiarrhythmic agent Arch Int Pharmacodyn 191 162 1971

19 Spurrell R. A J The effects of disopyramide on the human heart An electrophysiological study J Int Med Res (Suppl) 1 31 1976

20 Spurrell R A J Thorburn C W Camm J Sowton E & Deuchar D C Effects of disopyramide on electrophysiological properties of specialized conduction system in man and on accessory atrioventricular pathway in Wolff-Parkinson-White syndrome Br Heart J 37 861 1975

21 Vismara L Mason D & Amsterdam E Disopyramide phosphate Clinical efficacy of a new antiarrhythmic drug Clin Pharmacol Ther 16 330 1974

22 Weissler A Harris W & Schoenfeld C Systolic time intervals in heart failure in man Circulation 37 149 1968

23 Yeh B Sung P K & Scherlag B Effects of disopyramide on electrophysiological and mechanical properties of the heart J Pharm Sci 62 1924 1973



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Arrhythmias in Patients with Acute Cerebrovascular Disease

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ABSTRACT Cardiac disease is common in patients with cerebrovascular disease (CVD) and cerebral lesions as such may influence cardiac activity and rhythm To study the indication for continuous ECG surveillance of patients with CVD, 100 consecutive patients admitted to a medical stroke unit were investigated with 24-hour Holter recordings The patients' mean age was 73 years and 70% of them had a history of heart disease Twenty three patients had chronic atrial fibrillation and 55% of the remainder showed ventricular ectopic activity Serious ventricular arrhythmias were comparatively rare and mainly seen in association with signs of congestive heart failure and acute myocardial infarction A prolonged Q-T interval was registered in two-thirds of the patients but there was no significant association between this finding and ventricular ectopic activity Close observation for cardiac complications is important in patients with CVD and continuous ECG surveillance is indicated in selected high risk patients

Key words Cerebrovascular disease arrhythmias

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It is well known that cerebral disease may induce cardiac arrhythmias (1 13) In a clinical setting serious ventricular arrhythmias have been described particularly in association with subarachnoid haemorrhage (1 3 4 5) However indications for continuous ECG surveillance of patients with acute cerebrovascular disease (CVD) have not been agreed upon (12 14 16)

The aim of the present investigation has been to study the incidence and implications of arrhythmias in patients with CVD admitted to a non intensive stroke unit in a medical department

PATIENTS AND METHODS

In 1976 a unit for patients with CVD was arranged as part of an ordinary medical ward at Serafimerlasarettet Since 1977 the unit has had 10 beds Patients with suspect CVD admitted to the Casualty Department are transferred to the unit on a non-selective basis The patients are examined repeatedly during their stay in hospital and laboratory and X ray examinations are performed according to a strict schedule Clinical findings and liquor spectrophotometry have been the main basis for diagnosis (11) Relevant findings are registered in code on special charts for subsequent transfer to punch cards and tape for computer analysis Criteria for admission diagnosis and a description of the routines in the unit are presented elsewhere (2)

Routine 12 lead ECG recordings and enzymes (S-ASAT S-ALAT) and in most cases S-CK and S-LD were followed during the first three days after admission Holter ECG recordings (8) during 24 hours were made as soon as possible after admission to the unit. One hundred consecutive patients were studied 51 women and 49 men with a mean age of 73 years (range 51–94) The recordings were made 1–12 days (mean 2 4) after the onset of illness The tapes were read on an Avionics Composite Electrocardioscanner model 650 by two of the authors (C R and C H) Because of the difficulty in distinguishing between ventricular ectopic beats (VEB) and aberrantly conducted supraventricular beats in atrial fibrillation ventricular ectopic activity was not studied in patients with this arrhythmia

Q-T intervals were measured in a 12 lead ECG registered in association with the ECG recording Patients with atrial fibrillation and/or bundle branch block (BBB) were excluded Mean Q-T of three beats in succession was registered and the heart rate (RR interval) of the same beats was calculated Normal Q-T values women 0 43 men 0 42 sec Corrected Q-T interval (QT_c) was calculated by Bazett s formula

$$QT = \frac{QT}{\sqrt{RR}} \qquad (9)$$

RESULTS

Diagnoses and mortality

The final agreed clinical diagnoses are presented in Table I The ischaemic lesions dominated In the

Abbreviations CVD=cerebrovascular disease VEB=ventricular ectopic beats QT =corrected Q-T interval AMI=acute myocardial infarction BBB=bundle branch block

Table I *Diagnoses mortality previous heart disease and hypertension in the 100 patients studied*

	No of pats	No of deaths
Diagnosis		
Cerebral haemorrhage	8	3
Atherothrombotic infarction	62	10
Cerebral embolus	19	7
Transitory ischaemic attacks	9	
Other CVD	2	1
Total	100	21
Previous diseases		
Angina pectoris	16	
Myocardial infarction	11	
Heart failure	36	
Hypertension	36	

group with cerebral haemorrhage there was no patient with subarachnoid haemorrhage Total hospital mortality was 21%

Previous heart disease

The patients previous cardiac diseases are presented in Table I More than one third had a history of heart failure and another third of hypertension The patients who had no history of heart disease (30%) had a mean age of 72 years compared to 73 years in the whole series

Twenty one per cent of the patients were on digi- 25% on diuretics and 8% on other antihyper e drugs Ten patients had been taking β receptor blocking agents or verapamil before admission Mortality among patients with previous heart disease including those with chronic atrial fibrillation was 26% against 10% among those without such disease This difference did not quite reach statistical significance

Arrhythmias

Table II shows the incidence of different arrhythmias during the ECG recordings Eighteen per cent of the patients showed no arrhythmias or conduction disturbances at all during the 24-hour recording Twenty three patients mean age 79 years had chronic atrial fibrillation and two paroxysmal atrial fibrillation Except those with atrial fibrillation and a final diagnosis of cerebral embolus arrhythmias were not believed to have been the cause of focal neurologic deficit in any of the patients

Bradycardia was a rare finding The few patients concerned had usually been on digitalis The only patient with sinus arrest and nodal rhythm was a 79-year old man with an extensive atherothrombotic brain infarction who succumbed a few days after the recording Two patients one with a known first degree heart block and the other with intermittent Wenckebach periodicity were not on digitalis medication One patient had had a pacemaker for several years because of Stokes Adams attacks

Ventricular ectopic activity was registered in 42 (55%) out of 77 patients without chronic atrial fibrillation Ten of these patients had multifocal or coupled VEB Three of the patients had a verified acute myocardial infarction (AMI) during the ECG recording Two of them had multifocal VEB and one coupled VEB and repeated ventricular tachycardias All three patients died the latter in a cardiac rupture less than 24 hours after the ECG recording The incidence of VEB was not significantly increased in patients with previous heart disease as compared to those without such a history however all patients with chronic atrial fibrillation were excluded from this comparison Ventricular ectopic activity was not seen significantly more often in any kind of CVD

Thirteen patients had serum potassium levels below the normal range (3 6–5 2 mmol/l) on admission The lowest value was 3 1 mmol/l and the majority of these patients had been on diuretics Four of them showed VEB in the recording

Q T interval

Among the 100 patients, 23 with atrial fibrillation six with BBB and one with pacemaker were omitted

Table II *Incidence of arrhythmias during a 24 hour ECG recording*

	No of pats
Supraventricular ectopic beats	37
Paroxysmal tachycardia	3
Atrial fibrillation	23
Supraventricular bradycardia	2
Sinus arrest	1
Nodal rhythm	1
First degree heart block	3
Second degree heart block	2
Complete heart block	1
Left anterior hemiblock	2
LBBB	3
RBBB	1
Monofocal VEB	42
Multifocal and coupled VEB	10
Ventricular tachycardia	1

from the Q T calculations In one additional patient QT_c could not be calculated with accuracy because of the high frequency of ectopic beats Of the remaining 69 patients (36 men and 33 women) QT_c was increased in 46 (67%) In most of these patients the increase in QT_c was moderate >0 50 sec in only three

A prolonged QT_c was not seen significantly more often in patients with ventricular ectopic activity on the 24-hour recording than in those without such activity Increased QT_c was registered in all diagnostic groups of CVD it was not a more common finding in the small group with cerebral haemorrhage than in the others

In six of the 13 patients with a low serum potassium value on admission the Q T interval was increased Serum calcium was subnormal (normal value 2 20–2 60 mmol/l) in nine patients two of whom had an increased Q T time

DISCUSSION

Previous heart disease including hypertension was very common in the present group of patients with CVD admitted to a non intensive stroke unit in a medical department Only one third of the patients had no prior cardiac disease This finding is in agreement with results from other stroke units (12 16) and suggests that a considerable incidence of different arrhythmias may be expected in this patient group

In a population study of 70-year old people in Gothenburg Sweden (17), atrial fibrillation was registered in routine ECGs in 2% of the men and 4% of the women and VEB in 6 and 5% respectively A similar incidence of VEB has been reported in routine ECGs in survivors of myocardial infarction before discharge from hospital but in six hour ECG recordings VEB incidence increased to 72% in the same patients (15) a finding comparable to the incidence of VEB of 62% reported by Hinkle et al (7) in a study of 301 actively employed American men with a median age of 55 years

In a study by Norris and Hachinski (16) 50% of 103 stroke patients—the majority of whom were admitted within 10 hours of the onset of illness—showed arrhythmias during their stay in a stroke unit 12% had chronic atrial fibrillation 5% paroxysmal atrial fibrillation and about 25% ventricular ectopic activity Sinus bradycardia and nodal rhythm were rare findings and were usually registered in association with terminal coning

Only 18% of our patients were without any arrhythmias during the 24-hour recording The number of patients with chronic atrial fibrillation was remarkably high 23% Age may partly explain this finding the mean age of our patients with atrial fibrillation was as high as 79 years It may be mentioned that in a study from our hospital of 373 AMI patients mean age 66 years the total incidence of atrial fibrillation (including paroxysmal attacks) was 15% during the first 24 hours in the Coronary Care Unit (6)

As regards ventricular ectopic activity, a higher incidence was registered 55% in patients without chronic atrial fibrillation than in the study by Norris and Hachinski (16) Considering the related results of Hinkle et al (7) the high prevalence of heart disease and the high mean age of the present patient group the figure may hardly be regarded as unexpectedly high A surprising finding was that patients with previous heart disease did not have a significantly higher frequency of VEB than other patients However no conclusion regarding the causal relationship between the cerebral lesions and the arrhythmias can be drawn

More serious ventricular arrhythmias were registered in ten patients multifocal or coupled VEB or ventricular tachycardia Five of these patients had a disturbed consciousness on admission and thus belonged to a grave prognostic group All of these patients died Three of them had an AMI at the time of the ECG recording and one died an arrhythmic death Among the remaining five patients wit multifocal or coupled VEB three were in heart failure and received intensive treatment with digitalis and diuretics Thus only two patients without known heart disease showed more serious forms of ventricular arrhythmias in association with their stroke No antiarrhythmic treatment was given to these patients

It is accepted that the autonomic nervous system may influence the recovery properties of the ventricular myocardium (1) and that an increased liability to ventricular ectopic activity may be associated with an increased Q T interval (10) A tendency to a prolonged Q T interval has been described in patients with CVD and especially in those with subarachnoid or intracerebral haemorrhage (3 4 5) For this reason CT was studied in all patients without atrial fibrillation or BBB A prolonged Q T time was

registered in two-thirds of the present patient group but prolongation was usually moderate and there was no significant association between a long Q T time and the incidence of VEB A long Q-T interval was not a characteristic finding in any type of stroke

In conclusion it may be noted that a very high proportion of our patients showed some kind of arrhythmia However very few patients without known heart disease had serious ventricular arrhythmias Careful management of signs of heart failure and recognition of the possibility of concurrent AMI in stroke patients is important More close ECG surveillance of such selected high risk patients may also be of value General continuous ECG monitoring of stroke patients admitted to a medical department hardly seems indicated

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REFERENCES

1 Abildskov J A Millar K Burgess M O & Vincent W The electrocardiogram and the central nervous system Prog Cardiovasc Dis 8 210 1970
2 v Arbin M Britton M de Faire U Helmers C Miah K Murray V & Wester P O A stroke unit in a medical department Organization and the first 100 patients Acta Med Scand 205 231 1979
3 Burch G E Meyers R & Abildskov J A A new electrocardiographic pattern observed in cerebrovascular accidents Circulation 9 719 1954
4 Byer E Ashman R & Toth L A Electrocardiogram with large upright T waves and long Q-T intervals Am Heart J 31 796 1947
5 Hansson L & Larsson O The incidence of ECG abnormalities in acute cerebrovascular accidents Acta Med Scand 195 45 1974
6 Helmers C Short and long term prognostic indices in acute myocardial infarction Acta Med Scand (Suppl) 555 1973
7 Hinkle L E Jr Carver S T & Stevens M The frequency of asymptomatic disturbances of cardiac rhythm and conduction in middle aged men Am J Cardiol 24 629 1969
8 Holter N J New methods for heart studies Science 134 1214 1961
9 Hurst J Willis R & Bruce L The heart p 301 McGraw Hill New York 1970
10 Jervell A & Lange Nielsen F Congenital deaf mutism functional heart disease with prolongation of the *Q T interval and sudden death Am Heart J 54 59* 1957
11 Kjellin K G & Soderstrom C E Diagnostic significance of CSF spectrophotometry in cerebrovascular disease J Neurosurg Sci 23 359 1974
12 Lavy S Yaar, I Melamed E & Stern S The effect of acute stroke on cardiac functions as observed in an intensive stroke care unit Stroke 5 775 1974
13 Mauck H P Jr & Hockman C H Central nervous system mechanisms mediating cardiac rate and rhythm Am Heart J 74 96 1967
14 McHenry L C Toole, J F & Miller H S Long term EKG monitoring in patients with cerebrovascular insufficiency Stroke 7 264 1976
15 Moss A J Schnitzler R Green R & Decamilla J Ventricular arrhythmias 3 weeks after acute myocardial infarction Ann Intern Med 75 837 1971
16 Norris J W & Hachinski V C Intensive care management of stroke patients Stroke 7 573 1976
17 Svanborg A Seventy year old people in Gothenburg A population study in an industrialized Swedish city II General presentation of social and medical conditions Acta Med Scand (Suppl) 611 1977

Acta Med Scand 205 429–435 1979

Treatment and Prognosis in Polymyalgia Rheumatica and Temporal Arteritis

A Ten Year Survey of 53 Patients

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ABSTRACT The course of the disease and the effect of corticosteroid treatment were studied in 53 patients in whom polymyalgia rheumatica (PMR) or temporal arteritis (TA) had been diagnosed between 1967 and 1977 The mean interval between the onset of symptoms and confirmation of the diagnosis was 7 months The starting dosage of prednisolone was 30–40 mg daily for patients with evidence of cranial arteritis and 10–20 mg daily for those without The mean daily maintenance dose of prednisolone was 7 5 mg, usually 5 mg for patients with uncomplicated PMR and 10 mg for patients with TA Only 7 (16%) patients with symptoms due to PMR continued to feel well and had no relapse of symptoms on 2 5 mg prednisolone daily Corticosteroids were withdrawn from 19 patients 1 month to 4 years (mean 16 months) after the start of treatment By 1977, 34 patients had received prednisolone continuously for 8 months to 9 years (mean 3 years) Attempts to witdraw corticosteroids provoked 33 relapses in 20 patients, and 21 patients had a total of 33 relapses while on maintenance dosage In 5 of the latter a definite relapse of clinical symptoms was not accompanied by a concomitant rise in ESR No serious complications of the disease occurred in this series after the start of therapy, and complications attributable to treatment were infrequent The duration of the active stage of the disease varies widely It is rarely advisable, however, to end treatment with corticosteroids before the end of 1 year, and most patients need such treatment for at least 2–3 years

Key words polymyalgia rheumatica temporal arteritis corticosteroid treatment prognosis

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Polymyalgia rheumatica (PMR) and temporal arteritis (TA) (or giant cell arteritis) are diseases of old people that are moderately common self limiting and have a good prognosis (2 16) Considering that in these two diseases the sex and age distributions are the same the systemic symptoms are virtually identical and more than 50% of patients with uncomplicated PMR have arteritic lesions on biopsy (7 10 11) the two syndromes may well be different expressions of the same disease process

From reports on patient series in which PMR was followed but not treated it now appears that the average natural course of this disease is considerably longer than had been believed—2–4 years (5 8 21) or even 7 years (1) In rare instances PMR may persist for as long as 14 years Whereas the excellent response to corticosteroid treatment is a characteristic and an almost diagnostic feature of PMR there is no evidence that corticosteroids will shorten its course Corticosteroids administered in low dosage during the period of active disease will however keep most patients free from symptoms Recent reports (2 19) suggest that despite the theoretical danger associated with long term corticosteroid treatment in old people complications of such a regimen in PMR and TA are infrequent Hence long term administration of corticosteroids has been the treatment of choice

Since 1967 I have followed all patients diagnosed as having PMR or TA at the Fourth Department of Medicine Helsinki University Central Hospital This report analyses the findings over a 10-year period in this patient group Special attention has been focused on the mode of administration and duration of the corticosteroid therapy on the rate of relapses during and after withdrawal of corticosteroids and on complications and causes of death during treatment and follow up

Abbreviations PMR = polymyalgia rheumatica TA = temporal arteritis

Table I *Laboratory values before the start of treatment in 43 patients with PMR and 10 with TA w muscular symptoms (TA+PMR)*

	PMR		TA + PMR	
	Mean	Range	Mean	Range
Hb (g/100 ml)	11 6	7 7–14 6	10 2	7 5–12.5
ESR (mm/h)	88	54–133	103	81–130
α_2 globulin (g/100 ml)	0 99	0 5–1 4	1 4	0 6–1 5
Gammaglobulin (g/100 ml)	1 5	0 7–2 4	1 3	0 8–1 9

PATIENTS AND METHODS

The series comprised 53 patients 47 women and 6 men who between 1967 and 1977 had been diagnosed as having PMR or TA Their mean age at the time of diagnosis was 68 years (range 51–84) The clinical diagnosis was PMR in 43 patients and TA in 10 In all 10 patients who presented with symptoms due to TA symptoms typical of PMR also developed during the course of the disease

The diagnostic criteria were advanced age and a raised ESR plus in the case of PMR proximal muscular symptoms of at least 1 month s duration in the shoulder or pelvic girdles or in the case of TA local symptoms typical of TA or other evidence of cranial involvement and/or histological evidence of giant cell arteritis Progress was assessed by the patient s subjective feeling and by the rate at which the painful limitation of movements of the shoulder and hip—as well as the ESR—returned to normal Criteria for relapse of the disease were a) recurrence of the original symptoms usually but not always accompanied by a rise in the ESR b) a sharp rise in the ESR accompanied by a deterioration in the subjective condition but not by local symptoms and c) a complete relief of clinical symptoms and a drop in the ESR when corticosteroids were readministered or their dosage was increased

Table II *Initial and maintenance dosages of corticosteroids administered to 53 patients with PMR or TA*

Prednisolone dose (mg/day)		No of pats
Initial		
>45		1
35–45		5
20–30		25
<20		22
Mean	21 6	
Range	10–60	
Maintenance		
>10		7
5–10		39
<5		7
Mean	7 5	
Range	2.5–15	

RESULTS

Laboratory and biopsy findings

Table I shows the main laboratory findings befo the start of corticosteroid treatment In no patie was the ESR less than 54 mm/h Patients with cra al involvement had a slightly lower Hb value and slightly higher mean ESR and serum α_2 globul concentration than those with no such involvemer but this difference was not significant Twenty fi per cent of the patients had a platelet count high than 400000/mm³ The results of liver function tes performed in this series have recently been report (17) The activity of serum alkaline phosphatase mainly of hepatic origin was increased in 62% the patients and bromsulphalein retention was al normal in 46% Biopsies of the temporal arte performed in 20 patients revealed arteritis in 3 of t 11 patients with uncomplicated PMR and in 8 of t 9 with evidence of cranial involvement The resul of the fine needle liver biopsies performed in th series have been reported elsewhere (17)

Treatment and course of the disease

All 53 patients received corticosteroids in the for of prednisolone or methylprednisolone Indometh cin 25–50 mg daily given to several patients a supportive treatment was found to be very effecti against morning stiffness

The dosage schedules for corticosteroids varie with the type and severity of illness (Table II) Usually patients with evidence of TA were starte on 30–40 mg of prednisolone as a single mornin dose and patients with symptoms due to PMR o only 10–20 mg Only one patient presenting wit ophthalmoplegia received an initial dose of 60 m prednisolone daily Of the patients with PMR onl 7 felt well and had no relapse of symptoms on 2 mg prednisolone daily for lengthy periods Patient with cranial symptoms required a maintenance dos of up to 10 or 12 5 mg a day

Table III *Duration of treatment and follow up in 53 patients with PMR or TA*

	No of pats			Duration of treatment (mo)		Follow up after treatment (mo)	
	Total	PMR	TA	Mean	Range	Mean	Range
Corticosteroids withdrawn	19[a]	16	3	16	1–52	26	2–108
Corticosteroids continued	34[b]	27	7	36	8–108		

[a]2 deaths [b]6 deaths (Table VIII)

By the time of the last follow up it had been possible to withdraw corticosteroids in 19 patients (Table III) Only four of these patients had been followed for less than 1 year after completion of treatment Of the 10 patients no longer on corticosteroids who were seen at the last follow up 7 had normal ESR Hb level platelet count and serum alkaline phosphatase activity (Table IV) A review of the records of the other 9 patients no longer receiving corticosteroids—but not seen at last follow up—showed that clinical and laboratory remission had been complete in all At the last follow up 28 of the 34 patients still on prednisolone were alive (Table III) None of them showed any sign of recurrence of their original symptoms In all but 5 of these 28 patients laboratory values had returned to normal (Table IV) In 3 the ESR was more than 50 mm/h and 2 had a platelet count above 400 000/mm³

Relapses after withdrawal of corticosteroids

Attempts to withdraw corticosteroids at various stages of the disease provoked a total of 33 relapses of the rheumatic symptoms in 20 patients (Table V) Of the relapses 23 (71%) occurred in patients treated for less than 1 year However 6 patients treated for longer than 2 years also had a flare up of their symptoms when corticosteroids were withdrawn One patient whose original symptoms were due to TA with PMR had a severe flare up of muscular symptoms within 1 month after the termination of a 4 year course of treatment The interval between withdrawal of corticosteroids and relapse was less than 2 weeks in 18 (55%) of the relapses and longer than 1 month in 7 relapses One patient relapsed 2 years after the end of a 10-month course of treatment

Relapses during treatment with corticosteroids

While receiving corticosteroids 21 patients had a total of 33 relapses (Table VI) Three patients relapsed more than 3 years after the start of treatment In one whose original symptoms were due to PMR a transient hemiparesis and flare up of the muscle symptoms developed when the daily dose of prednisolone was decreased from 10 to 5 mg 4 years after the start of therapy In another patient whose symptoms were due to PMR and TA the muscular symptoms recurred 7 5 years after the start of treatment At relapse the muscular symptoms were almost always identical—both in type and location—to the patient s original symptoms Of 7 patients who presented with cranial symptoms 3 relapsed with similar symptoms and 4 had muscular symptoms only At the time of relapse the mean

Table IV *Laboratory values in the 38 patients with PMR or TA who were seen at the last follow up*

	Corticosteroids withdrawn (10 pats)		Still on corticosteroids (28 pats)	
	Mean	Range	Mean	Range
Hb (g/100 ml)	13 6	11 5–15 5	13 3	9 3–15 6
ESR (mm/h)	22	5–50	24	5–76
Platelets (× 10³/mm³)	309	234–512	328	200–1 030
Serum alkaline phosphatase[a] (U/l)	188	103–277	171	130–272

[a]Normal limit below 220 U/l

Table V Clinical relapses in 20 patients with PMR or TA after attempts to discontinue treatment with corticosteroids

	No of relapses
Duration of treatment (mo)	
1–12	23
13–24	4
25–36	4
>36	2
Mean 16	
Range 1–56	
Interval between withdrawal and relapse (weeks)	
1–2	18
3–4	8
>4 (1 5–24 mo)	7

daily maintenance dose of prednisolone was 7 3 mg for patients with PMR and somewhat higher for patients with evidence of cranial arteritis Among the patients with PMR 64% of the relapses occurred when the dosage of prednisolone was reduced from 5 to 2 5 mg daily among patients with TA 54% of the relapses were provoked by a reduction of the dosage from 7 5 to 5 mg daily

At relapse the laboratory profile closely resembled that observed before the start of therapy The mean ESR for the whole series however was lower at relapse in 5 patients who had a definite clinical relapse of symptoms the ESR did not rise above 30 mm/h

Complications of treatment and complicating disorders

Complications which could have been caused by corticosteroids were observed in 10 patients (Table

Table VI Clinical relapses in 21 out of 53 patients with PMR or TA during treatment with corticosteroids

Duration of treatment before relapse (mo)	No of relapses
1–6	14
7–12	7
13–24	7
25–36	2
>36	3
Mean 12	
Range 1–91	

Table VII Non fatal complications of tre[atment] and complicating disorders in 53 patients wi[th PMR] or TA during treatment with corticosteroids

	No [of] pat[ients]
Complications	
Related to corticosteroid therapy	
Diabetes mellitus	4
Severe spinal osteoporosis with vertebral fracture	2
Gluteal phlegmone with osteitis	1
Worsening of senile cataract	1
Cushingoid facial appearance	2
Possibly associated with rheumatic disease	
Thyrotoxicosis	1
Hypothyroidism	1
Disorders	
Cardiovascular	
Myocardial infaction	3
Reversible hemiparesis	1
Mesenteric arterial thrombosis	1
Malignant	
Breast carcinoma	1
Chronic lymphatic leukaemia	1

VII) In one patient thyrotoxicosis was associ[ated] with a relapse of the muscular symptoms and a [rise] in the ESR In another patient hypothyroidi[sm] with a high antithyroglobulin titre develope[d] months after the start of treatment Both patie[nts] who incurred fractures of a single lumbar vertel[bra] were more than 80 years old and had received co[rti]costeroids for 3 and 7 years, respectively Two [of] the 4 patients who developed diabetes requir[ed] treatment with insulin In none of the three patien[ts] who had a myocardial infarction was this associat[ed] with a flare up of the rheumatic symptoms O[ne] patient who was operated on for an acute mesente[r]ic arterial thrombosis had been taken off steroids [] months earlier No sign of an active arteritis wa[s] detected in the arterial biopsy specimen from th[e] resected intestine In 1 patient chronic lymphati[c] leukaemia was diagnosed 5 years after the onset o[f] PMR (15) and in another a non metastasizing car[c]inoma of the breast was surgically removed 3 years after the onset of PMR (14)

Causes of death

Of the 53 patients studied 8 had died by 1977 (Table VIII) Two of the deaths were due to cardiovascular complications after a short course of prednisolone

Table VIII *Causes of death in 8 patients with PMR or TA with muscular symptoms (TA + PMR)*

Pat no	Rheumatic disease	Duration of corticosteroid treatment (mo)	Primary cause of death
1	TA + PMR	2 (corticosteroids withdrawn 8 mo before death)	Intestinal infarction
2	TA + PMR	6 (corticosteroids withdrawn 2 mo before death)	Stroke
3	TA + PMR	66	Myocardial infarction
4	PMR	36	Pulmonary embolism Hodgkin s disease
5	TA + PMR	27	Metastasizing breast carcinoma
6	PMR	14	Pulmonary embolism congestive heart failure
7	TA + PMR	38	Myocardial infarction
8	TA + PMR	34	Stroke

treatment had been discontinued Autopsy was performed on one of these two and revealed an active myocarditis but no sign of arteritis Of the other 6 patients who died during treatment 4 had presented with cranial arteritis In none of these 4 did autopsy reveal either active or healed arteritis in the large aortic branches The 2 patients who died of fatal malignancies (nos 4 and 5) have been reported on elsewhere (16)

DISCUSSION

Although this series is comparatively small it includes patients with all the typical clinical features of PMR as well as patients in whom the close relation between PMR and cranial arteritis is clearly demonstrated

Of the non specific laboratory parameters used as indicators of disease activity the most reliable is still a considerably raised ESR In this series more over the magnitude of the increase in ESR and in the activity of serum alkaline phosphatases correlated strikingly with the severity of clinical symptoms (17) Only 23% of the temporal arterial biopsies taken from patients with pure PMR were positive a finding that agrees with the results of some studies (4 6) and contrasts with those of others (8 11) reporting arteritic changes in about 50% of biopsies from patients with uncomplicated PMR However because the distribution of arteritic changes is segmental (10 11) temporal arterial biopsies may sometimes be negative even in patients whose cranial symptoms strongly suggest active arteritis This was the case with one patient in the present study Biopsy of a temporal artery should always be performed in patients with cranial symptoms but seems unnecessary in patients with muscular symptoms only

The great differences among PMR patients in the duration of the active stage of the disease has led to some confusion about the mode of administration and the required duration of corticosteroid treatment Some experienced clinicians have recommended higher starting doses of corticosteroids than those reported here (8 10) However my experience has shown in agreement with Myles (20) that an adequate starting dose for most patients with TA who have no ocular complications is 30–40 mg prednisolone daily and for patients with uncomplicated PMR 10–20 mg The mean maintenance dosages of prednisolone I administered were similar to those reported in most other studies

The recently recommended minimum duration of treatment in PMR and TA is 2 years (8 24) In the present study the mean duration of treatment among the patients who had been taken off corticosteroids was 16 months and among those who had received corticosteroids continuously 3 years That some patients need prolonged treatment with corticosteroids—for 5 years or more (20) or for over 9 years as did one of my patients—is evidence of the long period of active disease in such patients

Thirty five per cent of the patients had a clinical relapse during the course of treatment an incidence somewhat higher than that reported by Fauchald et al (8) but lower than that reported by Paulsen and Iversen (21) That the reports on the incidences of relapses differ may be due to differences in such factors as the duration of treatment the criteria for a clinical relapse and the schedule for reducing the dose of steroids A too rapid reduction of the starting dose might lead to an early flare up of

toms (10 13) Relapses may occur however even during a slow tapering of the initial (2) or maintenance (7) dose of corticosteroids The results of this study as well as of that by Esselinckx et al (7) suggest that withdrawal of corticosteroids while the disease is still active will lead to a relapse of symptoms in about 80% of patients If 1–2 months after discontinuation of treatment the patients is still symptom free and the ESR has not risen then the disease is probably no longer active Because a sizeable number of relapses occur some months after the end of treatment—as shown in this and other studies (8 19)—patients dismissed from treatment should for at least 1 year thereafter, have periodic check ups that include measurement of the ESR It must be remembered moreover that a relapse may be characterized by a recurrence of symptoms with no re-elevation of the ESR (3 7 23) as well as by a sharp rise in the ESR unaccompanied by local symptoms (2)

In this series the complications attributable to corticosteroid treatment were relatively benign and similar to those reported by others (4 19 20) That 2 patients developed thyrotoxicosis and hypothyroidism respectively and that 5 patients had a history of hypothyroidism is interesting in view of the recently observed association between thyroid dysfunction and giant cell arteritis (25) The high in‑ of fatal and non fatal cardiovascular com‑ in this series is equal to that reported for comparable series and is not necessarily either to active arteritis or to treatment with corticosteroids The incidence of malignant diseases did not differ appreciably from that anticipated among persons of the same age in the general population (16) I agree with Myles (20) that malignant disorders and chronic infections seldom present problems in the differential diagnosis In this series such problems occurred only with rheumatoid arthritis

In summary My findings show (*a*) that long term treatment of PMR with corticosteroids is safe for most patients (*b*) that an adequate starting dose of prednisolone for patients with uncomplicated PMR is 10–20 mg and for those with cranial symptoms 30–40 mg—with higher starting doses required only for patients with ocular complications (*c*) that daily maintenance doses lower than 5 mg prednisolone in PMR and 7 5–10 mg in TA increase the risk of relapse (*d*) that most patients need treatment for at least 2–3 years and hence withdrawal of corticosteroids before the lapse of 1 year is not advisable and (*e*) that owing to the risk of late relapses, patients taken off steroids should have periodic clinical and laboratory check ups for at least 1 year after the end of treatment

REFERENCES

1 Bagratuni L Prognosis in the anarthritic rheumatoid syndrome Br Med J 1 513 1963
2 Beevers D G Harpur J E & Turk K A D Giant cell arteritis—the need for prolonged treatment J Chron Dis 26 571 1973
3 Bruk M J Articular and vascular manifestations of polymyalgia rheumatica Ann Rheum Dis 26 103 1967
4 Coomes E V Ellis R M & Kay G A prognostic study of 102 patients with the polymyalgia rheumatica syndrome Rheumatol Rehabil 15 270 1976
5 Davidson S Spiera H & Platz C M Polymyalgia rheumatica Arthritis Rheum 9 18 1966
6 Dixon A St J Beardwell C Kay A Wanka J & Wong Y T Polymyalgia rheumatica and temporal arteritis Ann Rheum Dis 25 203 1966
7 Esselinckx W Doherty S M & Dixon A St J Polymyalgia rheumatica Abrupt and gradual withdrawal of prednisolone treatment clinical and laboratory observations Ann Rheum Dis 36 219 1977
8 Fauchald P Rygvold O & Øystese B Temporal arteritis and polymyalgia rheumatica Clinical and biopsy findings Ann Intern Med 77 845 1972
9 Gordon I Polymyalgia rheumatica A clinical study of 21 cases Q J Med 29 473 1960
10 Hamilton C R Jr Shelley W M & Tumulty P A Giant cell arteritis Including temporal arteritis and polymyalgia rheumatica Medicine 50 1 1971
11 Hamrin B Polymyalgia arteritica Acta Med Scand (Suppl) 533 1972
12 Hunder G Disney T & Emmerson L Polymyalgia rheumatica Mayo Clin Proc 44 849 1969
13 Klein R G Hunder G G Stanson N W & Sheps S G Large artery involvement in giant cell (temporal) arteritis Ann Intern Med 83 806 1975
14 v Knorring J & Selroos O Sarcoidosis with thyroid involvement polymyalgia rheumatica and breast carcinoma Scand J Rheumatology 5 77 1976
15 — Polymyalgia rheumatica and chronic lymphatic leukaemia Scand J Rheumatology 6 145 1976
16 v Knorring J & Somer T Malignancy in association with polymyalgia rheumatica and temporal arteritis Scand J Rheumatol 3 129 1974
17 v Knorring J & Wasastjerna C Liver involvement in polymyalgia rheumatica Scand J Rheumatol 5 197 1976
18 Meadows S P Temporal or giant cell arteritis Br J Hosp Med 1 835 1967
19 Mowat A G & Hazleman B L Polymyalgia rheumatica—a clinical study with particular reference to arterial disease J Rheumatol 1 190 1974
20 Myles A B Polymyalgia rheumatica and giant cell

arteritis A seven year survey Rheumatol Rehabil 14 231 1975
21 Paulsen S & Iversen Th O Rheumatic polymyalgia Long term treatment with steroids Acta Rheumatol Scand 17 165 1971
22 Russell R W R Giant cell arteritis Review of 35 cases Q J Med 28 471 1959
23 Rynes R J Mika P & Bartholomew L E Development of giant cell (temporal) arteritis in a patient adequately treated for polymyalgia rheumatica Ann Rheum Dis 36 88 1977
24 Solberg Sørensen P & Lorenzen I Giant-cell arteritis temporal arteritis and polymyalgia rheumatica Acta Med Scand 201 207 1977
25 Thomas R D & Croft D N Thyrotoxicosis and giant-cell arteritis Br Med J 2 408 1974

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Thrombotic Thrombocytopenic Purpura
Treatment with a Combination of Antiplatelet Drugs

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ABSTRACT. Thrombotic thrombocytopenic purpura (TTP) is a rare disease with a very high mortality. Different modalities of therapy have been tried, but often with no effect. Recently, interest has focused on drugs interfering with platelet function, though few patients have received antiplatelet drugs as the only therapy. We describe a patient with TTP, who recovered completely on a combination therapy with dextran, aspirin and dipyridamole.

Key words: thrombotic thrombocytopenic purpura, antiplatelet drugs, full recovery.

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Thrombotic thrombocytopenic purpura (TTP) was first described in 1925 by Moschcowitz (18). Since then, more than 300 cases have been reported in the literature. The characteristic features of the syndrome are intravascular haemolysis, thrombocytopenia and neurological manifestations. Histology shows disseminated thrombi in arterioles and capillaries. The changes are often found in the kidneys, the pancreas and the CNS, but may occur in all organs. In most cases the disease takes a rapid and fatal course (2).

The aetiology and pathogenesis of TTP are still unknown, a fact that probably adds to the diversity of opinion on the therapeutic approach. Rubinstein et al. (19) reported on exchange transfusion, and haemodialysis was introduced by Tartaglia and Burkart (21); furthermore, heparin (5), dextran (16), plasmapheresis (6), vincristine (1) and splenectomy either alone or in combination with high doses of glucocorticoids (14) have been tried.

In 1972 Schwartz et al. (20) reviewed the number of long term survivors with TTP and found 32 cases, 23 of whom have been treated with a combination of glucocorticoids and splenectomy. Recently, interest has focused on drugs interfering with platelet function, e.g. dipyridamole and aspirin. However, the majority of patients who have obtained a remission on this therapy also underwent splenectomy and were treated with steroids (2).

The present study describes a case of TTP successfully treated with a combination of aspirin, dipyridamole and dextran without any other therapy. The aim of the therapy was to achieve maximal inhibition of the platelet function.

CASE REPORT

A 47-year-old man was admitted from a local hospital to the Division of Haematology, Hvidovre Hospital, after one week's complaints of moderate fever, night sweating and abdominal pain. The patient was anaemic with numerous petechiae. Blood pressure was 140/90 mmHg, pulse rate 84/min and the temperature 36.5°C. His general condition was rather good and the physical examination, including neurological examination, was normal. A blood status revealed a Hb concentration of 93 g/l with slightly elevated erythrocyte volume and a normal erythrocyte Hb concentration. The red cell volume fraction was 0.28 and the reticulocyte fraction 0.05.

Leukocyte count was 6.6×10^9/l with 2% myelocytes, 2% metamyelocytes, 64% neutrophil polymorphonuclear granulocytes, 22% lymphocytes and 3% monocytes. A blood smear showed poikilo- and anisocytosis with numerous schistocytes and burr cells (Fig. 1). Platelet count was 39×10^9/l. Bone marrow biopsy showed erythroid hyperplasia and an elevated number of megakaryocytes. Serum bilirubin was 28 µmol/l (5–17), serum lactic dehydrogenase 35.6 µkat/l (2.5–7.5), glutamic oxalacetic transaminase and albumin were normal. Serum creatinine was 142 µmol/l, serum urea 10.3 µmol/l, haptoglobin 1 µmol/l (5–37) and plasma Hb 5 µmol/l (0–14).

The coagulation parameters were as follows: activated partial thromboplastin time 3 arb. units (2.1–3.8), prothrombin time 0.94 arb. units (0.7–1.3), plasma fibrinogen 12.2 µmol/l (6–13), plasma antithrombin III 6.4 µmol/l (4.6–6.7), ethanol gelation test positive, fibrin degradation products 60 arb. units (0–5), plasma plasminogen 3 µmol/l (1.4–3.0), primary plasmin inhibitor 1.4 µmol/l (approx.

Abbreviations: TTP = thrombotic thrombocytopenic purpura, DIC = disseminated intravascular coagulation.

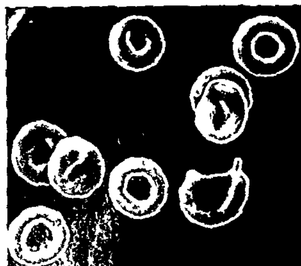


Fig 1 Scanning electron micrograph (provided by Dr P Christoffersen) of erythrocytes from peripheral blood showing variation in shape (×1200)

10) euglobulin lysis time more than 120 min (more than 60) and the Ivy bleeding time more than 15 min (1–5). *Figures given in parentheses above indicate reference values*

Coomb s test Donath Landsteiner Ham-Crosby and sucrose haemolysis tests were all negative There were no antinuclear factor or LE cell phenomenon The urine contained traces of protein but no Hb Urinary sediment was normal

Five days after admission the patient developed left sided hemiparesis and became lethargic and confused. Remission of the paresis was seen in a few hours but lethargy and confusion persisted A diagnosis of TTP was made on the basis of clinical features and laboratory data. Two skin biopsies were taken but both were normal

A trial of 80 mg of prednisone daily was instituted without any effect and was discontinued after 7 days At this time dextran infusions were started with 500 ml of dextran (MW 70000 6% in glucose) every 12th hour resulting in remission of the neurological symptoms within 24 hours. Only minor improvement in the platelet and reticulocyte concentrations was observed As the neurological symptoms reappeared aspirin 500 mg every 8 hours and dipyridamole 100 mg every 6 hours were added On this combined therapy with high molecular weight dextran aspirin and dipyridamole a clinical and laboratory remission was seen within 72 hours The bleeding time was longer than 15 min in spite of a normal platelet concentration No side-effects were noted

Discontinuation of dextran after 4 days of treatment resulted in a decrease in platelet concentration and an increasing reticulocyte concentration This relapse responded promptly when dextran was reinstituted Therapy with all three drugs was continued for another 12 days after which dextran was withdrawn This time no signs of relapse were seen and the patient was discharged on aspirin and dipyridamole 39 days after admission Dipyridamole and aspirin were withdrawn 50 and 70 days respectively after start of the treatment The physical examination and laboratory data are still normal one year after discharge The course of the disease is illustrated in Fig 2

DISCUSSION

Despite the lack of histological proof of TTP we consider that the clinical symptoms and laboratory data (microangiopathic haemolytic anaemia thrombocytopenia nephropathy and neurological symptoms) found in our patient indicate the presence of TTP

The pathogenesis of TTP remains controversial The occluding material consists of fibrin and platelets (4 10) and recent studies including immunofluorescence and electron microscopy have supported the hypothesis of a damaged vessel wall as a central feature of TTP which could be the primary event with secondary platelet adhesion and aggregation (9) Based on these considerations it seems rational to apply drugs interfering with platelet function

Disseminated intravascular coagulation (DIC) has been considered the primary pathological event based on the successful treatment with heparin in some cases (8) However the general opinion is that no DIC is present in typical TTP (15) No signs

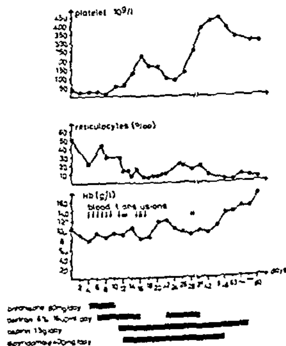


Fig 2 Clinical course during treatment.

of acute fulminant DIC or systematically increased fibrinolysis were found in our patient

The clinical course demonstrated that the combination of the three drugs was necessary in our patient and it is conceivable that this reflects the different sites of action of the three drugs Dipyridamole will raise the intracellular level of cyclic AMP by inhibiting the phosphodiesterase (17) which is well correlated to a decreased aggregation potential (23) Aspirin inhibits the enzyme cyclo-oxygenase (11) and thereby the synthesis of prostaglandin endoperoxides and the thromboxanes both of which can directly induce platelet release and aggregation (12 13) The action of dextran is less well understood but might be related to alterations in the platelet membrane (22)

Based on these phenomena we think that negative results following treatment of TTP with antiplatelet drugs mostly a combination of aspirin and dipyridamole might be due to insufficient inhibition of platelet function We are not aware of any other attempts with similar therapy as intense as that reported here

Only a few cases of relapse of TTP in long term survivors have been reported (7) This underscores the transient nature of the aetiologic process but as long as this is not understood the therapy should aim to inhibit the platelet thrombus formation We consider that a combination of antiplatelet drugs is promising in that respect and would prefer this therapy as first choice instead of a splenectomy which is risky in TTP patients

REFERENCES

1 Abramson N Treatment of thrombotic thrombocytopenic purpura Plasma vincristine hemodialysis and exchange transfusions N Engl J Med 298 971 1978
2 Amorosi E L & Karpatkin S Antiplatelet treatment of thrombotic thrombocytopenic purpura Ann Intern Med 86 102 1977
3 Amorosi E L & Ultmann J E Thrombotic thrombocytopenic purpura Report of 16 cases and review of the literature Medicine 45 139 1966
4 Baehr G Klemperer P & Schifrin A An acute febrile anemia and thrombocytopenic purpura with diffuse platelet thromboses of capillaries and arterioles Trans Assoc Am Physicians 51 43 1936
5 Bernstock L & Hirson C Thrombotic thrombocytopenic purpura Remission on treatment with heparin Lancet I 28 1960
6 Bukowski R M King J W & Hewlett J S Plasmapheresis in the treatment of thrombotic thrombocytopenic purpura Blood 50 413 1977
7 Cahalane S F Late recurrence of thrombotic thrombocytopenic purpura Br Med J 2 562 1975
8 Ekberg M Nilsson I M & Denneberg T Coagulation studies in hemolytic uremic syndrome and thrombotic thrombocytopenic purpura Acta Med Scand 196 373 1974
9 Feldman J D Mardiney M R Unanue E R & Cutting H The vascular pathology of thrombotic thrombocytopenic purpura Lab Invest 15 927 1966
10 Graig J M & Gitlin D The nature of hyaline thrombi in thrombotic thrombocytopenic purpura Am J Pathol 33 251 1957
11 Hamberg M Svensson J & Samuelsson B Mechanism of the anti aggregating effect of aspirin on human platelets Lancet 2 223 1974
12 — Thromboxanes A new group of biologically active compounds derived from prostaglandin endoperoxides *Proc Natl Acad Sci USA 72 2994 1975*
13 Hamberg M Svensson J Wakabayashi T & Samuelsson B Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation Proc Natl Acad Sci USA 71 345 1974
14 Hill J B & Cooper W M Thrombotic thrombocytopenic purpura Treatment with corticosteroids and splenectomy Arch Intern Med 122 353 1960
15 Jaffe E A Nachman R L & Merskey C Thrombotic thrombocytopenic purpura—Coagulation parameters in twelve patients Blood 42 499 1973
16 Lerner R G Rapaport S I & Meltzer J Thrombotic thrombocytopenic purpura Serial clotting studies relation to generalized Schwartzman reaction and remission after steroid and dextran therapy Ann Intern Med 66 1180 1967
17 Mills D C B & Smith J B The influence on platelet aggregation of drugs that affect the accumulation of adenosine 3 5 -cyclic monophosphate in platelets Biochem J 121 185 1971
18 Moschcowitz E An acute febrile pleiochromic anemia with hyaline thromboses of the terminal arterioles and capillaries An undescribed disease Arch Intern Med 36 89 1925
19 Rubinstein M A Kagan B M Macgillviray M H Merliss R & Sacks H Unusual remission in a case of thrombotic thrombocytopenic purpura syndrome following fresh blood exchange transfusions Ann Intern Med 51 1409 1959
20 Schwartz J Rosenberg A & Cooperberg A A Thrombotic thrombocytopenic purpura Successful treatment of two cases Can Med Assoc J 106 1200 1972
21 Tartaglia A P & Burkart P T Thrombotic thrombocytopenic purpura Remission following hemodialysis JAMA 218 999 1971
22 Weiss H J The effect of clinical dextran on platelet aggregation adhesion and ADP release in man In vivo and in vitro studies J Lab Clin Med 69 37 1967
23 — Platelet physiology and abnormalities of function N Engl J Med 293 531 and 580 1975

Sarcoidosis Presenting with Diabetes Insipidus Followed by Acute Cranial Nerve Syndrome

A Case Report

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ABSTRACT Diabetes insipidus in a previously healthy 16-year old girl led to surgical exploration of a pituitary stalk intumescence detected by oxygen cisternography with the use of tomography Biopsy of the pituitary stalk contained chronically inflamed brain tissue Subsequent liver and bone biopsies showed characteristic granulomata confirming the diagnosis of sarcoidosis Subfrontal craniotomy was followed by a rapidly progressive basal meningo-encephalitis with multiple cranial nerve involvement The need to establish a causal diagnosis in diabetes insipidus is stressed The rarity of the disorder and the presumed role of subfrontal craniotomy with regard to the flare up of the sarcoidosis of the brain are discussed

Key words sarcoidosis diabetes insipidus cranial nerve syndrome

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Approximately 5% of all sarcoidosis patients show involvement of the central nervous system (12 13 16 17 20) Although the process may be disseminated all over the brain and spinal cord the hypothalamic area and cranial nerves appear to be predilection sites for the granulomata (20) Rarely and presumably after prolonged existence the inflammation may give rise to mass lesions acting as brain tumours

In this paper a case of sarcoidosis is reported presenting with diabetes insipidus caused by a pea size pituitary stalk tumour in an otherwise healthy female adolescent Surgical exploration was followed acutely by serious impairment of several cranial nerves

CASE REPORT

A previously healthy 16-year-old girl presented with polyuria and polydipsia in April 1976 Six weeks before she had had an influenza like syndrome The diagnosis of diabetes insipidus was made and treatment with Minrin® (1-deamino-8-D-arginine vasopressin) was successful The patient was first admitted in Aug 1976 The history offered no clues to previous head injury tuberculosis or other infectious disease except the bout of influenza in February Dependence on Minrin and a vague frontal headache were the only complaints The existence of diabetes insipidus resulting from ADH deficiency could easily be confirmed Physical examination revealed no abnormalities Sexual development (Tanner's stage IV) was normal Bone age was consistent with 16 years There was no galactorrhoea X rays of the chest and sellar region were perfectly normal

Laboratory investigation revealed normal ESR normal blood count and morphology normal serum proteins urea nitrogen and creatinine calcium alkaline phosphatase bilirubin ASAT ALAT and normal excretion of bromsulphthalein The functions of the anterior pituitary were tested free thyroxine index was normal basal morning prolactin was elevated (54 ng/ml) LH RH injection was followed by a normal FSH increase and a pronounced rise of LH insulin provoked hypoglycaemia resulted in an adequate increase in both cortisol and growth hormone Ophthalmological examination gave no indication of uveitis visual field impairment or any other abnormality Findings at neurological examination were normal In the search for a possible sellar or suprasellar cause of the diabetes insipidus and hyperprolactinaemia bilateral carotid arteriography was done and showed no abnormalities Pneumoencephalography-cisternography using tomography strongly suggested a 5×5 mm intumescence of the pituitary stalk (Fig 1) The cerebrospinal fluid had a normal cell count glucose and protein content A tuberculin (PPD) skin test as well as serological tests for brucellosis and toxoplasmosis were negative

In Nov 1976 the patient was readmitted for follow up She still had the same complaints of vague supraorbital headaches and still needed Minrin She menstruated regu

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Fig 1 Lateral midline (*a*) and antero-posterior (*b*) tomograms during combined oxygen pneumoencephalography-cisternography demonstrates a small expanding lesion in the pituitary stalk

larly and had been attending school normally Findings at general physical and neurological examinations were as normal as before Visual field testing however raised suspicion of slight bitemporal upper field defects. The eyes were otherwise normal The air studies were repeated and gave exactly the same picture as in August without signs of progression Cerebrospinal fluid was again found to be normal Anterior pituitary function tests gave normal results with the exception of a definitely subnormal TSH response to TRH injection The free thyroxine index however was unchanged It was concluded that surgical exploration would offer the best chances for adequate diagnosis and in case of tumour for early treatment Subfrontal craniotomy was performed on Dec 24 The pituitary stalk was thickened corresponding with the pneumoencephalographic picture In a biopsy chronically inflamed brain parenchyma was seen with an extensive lymphocytic infiltrate containing also plasma cells and mast cells No indication of tumour fungal infection or tuberculosis could be found

Postoperatively the patient was very apathetic and drowsy The diabetes insipidus did not change and could be adequately controlled Five days after surgery bilateral peripheral facial palsy was noted A rapidly progressive perceptive loss of hearing was observed on the left and less severely on the right There was no vestibular disturbance The subsequent development of polyneuropathy in both legs was shown by serious impairment of tendon reflexes and sensibility Loss of the masseter reflex with facial sensory impairment indicated trigeminal involvement Diplopia was caused by left trochlear palsy Because of the histological description of the surgical biopsy and the postoperative clinical picture sarcoidosis of the central nervous system was considered the most likely diagnosis Skin tests for atypical mycobacteria were negative After biopsies from the liver and iliac crest and after a normal ^{99m}Tc bone scan prednisone therapy (30 mg daily) was initiated four weeks after operation Several well developed non necrotizing granulomata were seen in the liver and bone biopsy specimens yielding the convincing evidence for the existence of sarcoidosis

Within a few days after the start of prednisone therapy a remarkable improvement of the general condition was noted The patient regained alertness and activity Recovery of the facial palsy took several months Slight trochlear paresis and sensory loss in face and legs still persisted after 10 months Visual fields became normal except for slight enlargement of the blind spots Diabetes insipidus remained unchanged Both conventional audiometry and electrocochleography combined with acoustically evoked brainstem responses showed complete normalization of hearing on the right side but persistent damage to the innervation of the basal part of the left cochlea Because of development of symptoms of hypothyroidism and a subnormal free thyroxine index thyroxine replacement was given Prolactin remained elevated also after restoration of the euthyroid condition and small amounts of milk could be expressed from the breast Since the operation the patient did not menstruate

Bromocriptin® (2.5 mg three times daily) normalized the serum prolactin level and stopped galactorrhoea but did not restore menstrual cycles Like the absence of LH and FSH response to LH RH the amenorrhoea was ascribed to the corticoid therapy which is still being given.

DISCUSSION

Diabetes insipidus as the only presenting symptom of sarcoidosis is extremely rare The results of endocrine testing and local exploration of the sellar and suprasellar region by adequate X ray studies and subsequent surgery in this case stress the im-

portance of these efforts for the explanation of diabetes insipidus

Although a minor proportion of cases remain idiopathic even after thorough clinical testing (14) it will mostly be possible to find a tumour or inflammatory process as a cause if local surgery or head injury cannot be indicted Surgical exploration was thought to be indicated in our patient since the pituitary stalk thickening could represent a tumour (craniopharyngioma meningioma glioma teratoma a o) a granuloma (histiocytosis X sarcoidosis tuberculosis brucellosis, fungal or protozoan infection) a cyst or an extension of a pituitary adenoma (3 4 5) Early tumour extirpation could be complete and non surgical therapeutic measures would be hazardous without more precise knowlege about the nature of the abnormal structure It was due to the diabetes insipidus that this small focus of sarcoidosis was detected Localized sarcoidosis of the brain sometimes presents with larger conglomerations of granulomata acting like brain tumours (12 15) More often sarcoidosis inflicting the central nervous system manifests itself as chronic meningoencephalitis and this seldom occurs without previous uveitis parotitis pulmonary or skin lesions (4 6 8 9) Still more unusual was the strikingly acute postsurgical deterioration of this patient suffering from rapidly progressive basal meningoencephalitis Before surgery no neurological symptoms or cerebrospinal fluid abnormalities existed which could have warned and during subfrontal exploration no other visible abnormalities were encountered than the thickened pituitary stalk Very few cases of sarcoidosis involving the central nervous system in which craniotomy was followed by worsening are mentioned in the literature (6 12 15) The reason for the flare up is unclear If it is accepted that sarcoidosis may be an unfavourable immune response (8) to an as yet unknown and possibly non specific stimulus it might be argued that some existing equilibrium was disturbed Surgery possibly meant stress or spilling of antigen and implicated the administration of anaesthetics and a high dosage of hydrocortisone (to protect against ACTH deficiency as a possible result from pituitary stalk surgery) which was tapered off in a few days It is known that lowering of corticoid dosage in systemic *lupus erythematosus* or rheumatoid disease bears the risk of acute exacerbation or the onset of vascular manifestations (11 18) A bout of influenza like this patient had shortly before the onset of her diabetes insipidus is described more often as preceding deterioration of existing sarcoidosis (2 6 10) It is generally assumed that involvement of the central nervous system is a relatively late manifestation (6) In this case however nothing except the flu indicated possible previous disease Only when the patient deteriorated after craniotomy were liver and bone biopsies taken and revealed generalized disease In a similar future case liver biopsy might be considered earlier (1)

Spontaneous recovery from sarcoidosis is well known From the literature it can be concluded that corticoid treatment of sarcoidosis of the nervous system may give at least symptomatic improvement hopefully also preventing permanent damage (6 7) As far as already existent neuronal lesions are concerned no complete recovery can be expected In our patient partial improvement has been observed The rapid and steady recovery after corticoid administration suggests a favourable therapeutic effect The central loss of hearing of the left ear seems to be definitive Also because of the reported relapses after corticoid withdrawal this therapy will be continued for at least 2 years at the lowest effective dosage

REFERENCES

1 Beard W Foster D B Kepes J J & Guillan R A Xanthomatosis of the central nervous system Neurology (Minneap) 20 305 1970

2 Borner E Lofgren Syndrom (akuter Morbus Boeck) mit Polyneuritis Dtsch Med Wochenschr 93 1654 1968

3 Clinicopathological Conference Case 38 1975 N Engl J Med 292 653 1975

4 Colover J Sarcoidosis with involvement of the nervous system Brain 71 451 1948

5 Crompton M R In Pituitary tumours (ed J S Jenkins) chapter 2 Butterworths London 1973

6 Douglas A C & Maloney A F J Sarcoidosis of the central nervous system J Neurol Neurosurg Psychiatry 36 1024 1973

7 Findley L J Relapsing meningo-encephalitis Cerebral sarcoidosis? Proc R Soc Med 68 22 1975

8 Geraint James D Neville E & Langley D A Ocular sarcoidosis Trans Ophthalmol Soc UK 96 133 1976

9 Geraint James D & Sharma O P Neurological complications of sarcoidosis Proc R Soc Med 60 1169 1967

10 Henkind P & Gottlieb M B Bi ophthalmoplegia in a patient with sarco Ophthalmol 57 792 1973

some patients with asthma (14 18) This bronchoconstrictor effect can be blocked by atropine and by cooling the vagal nerves indicating a reflex reaction mediated through the vagi initiated by irritation of the cough receptors in the tracheobronchial tree (5 14 18 19)

Inhalation of disodium cromoglycate powder implicates local irritation and some subjects with asthma react with an increase in airway resistance the individual response being variable and difficult to predict (4 8 9) Disodium cromoglycate powder is in principle a foreign body in the lungs and pulmonary granulomatosis with giant cell formation has been observed in one case during long term treatment (3)

In the present case reflex bronchoconstriction was the likely cause of the severe reaction The patient had intrinsic asthma and no allergy to disodium cromoglycate was demonstrated

Sometimes inhalation therapy can provoke or deteriorate asthma in susceptible subjects the relation often being difficult to recognize There have been a few reports of asthma associated with adrenergic aerosols and of bronchial reactions to inhalant vehicles (2 6 10 12 17)

The increase in asthma mortality in Britain has been correlated to the extensive use of inhalation therapy (16) Inhalation therapy should therefore always be given to the problematic asthmatic patient with much awareness and some scepticism especially at exacerbations

REFERENCES

1 Altounyan R E C Variation of drug action on airway obstruction in man Thorax 19 406 1964
2 Bryant D H & Pepys J Bronchial reactions to aerosol inhalant vehicle Br Med J 1 1319 1976
3 *Burgher L W Kass I & Schenken J R Pulmonary allergic granulomatosis a possible drug reaction in a patient receiving cromolyn sodium* Chest 66 84 1974
4 Charpin J Gayrard P & Orlando J P Disodium cromoglycate (DSCG) Studies of lung function—clinical results In New concepts in allergy and clinical immunology (ed U Serafini A W Frankland C Masala & J M Jamar) p 392 Excerpta Medica, Amsterdam 1971
5 Dautrebande L Lovejoy F W & McCredie R M New studies on aerosols—effects of atropine microaerosols on the airway resistance in man Arch Int Pharmacodyn Ther 139 198 1962
6 *Fawcett I W Pepys J & Erooga M A Asthma* due to glycyl compound' powder—an intermediate in production of salbutamol Clin Allergy 6 405 1976
7 Freedman B J Bronchodilator aerosols Lancet 2 838 1967
8 General Practitioner Research Group report no 107 Disodium cromoglycate in asthma Practitioner 203 220 1969
9 — Report no 151 Disodium cromoglycate with and without isoprenaline Practitioner 205 230 1970
10 Keighley J F Iatrogenic asthma associated with adrenergic aerosols Ann Intern Med 65 985 1966
11 Muller J & Kowalski J Unusual side effects during disodium cromoglycate (Intal) therapy in a case of bronchial asthma Pneumologie 151 323 1975
12 Plaut G S Bronchodilator aerosols Lancet 2 771 1967
13 Scherrer M Die Stellung von Cromolyn (Lomudal Intal Aarane) im Behandlungsplan des Bronchialasthmas Pneumologie 151 1 1974
14 Simonsson B G Jacobs F M & Nadel J A Role of autonomic nervous system and the cough reflex in the increased responsiveness of airways in patients with obstructive airway disease J Clin Invest 46 1812 1967
15 Smith J M & Pizarro Y A Observations on the safety of disodium cromoglycate in long term use in children Clin Allergy 2 143 1972
16 Speizer F E Doll R Heaf P & Strang L B Investigations into use of drugs preceding death from asthma Br Med J 1 339 1968
17 Sterling G M & Batten J C Effect of aerosol propellants and surfactants on airway resistance Thorax 24 228 1969
18 Widdicombe J G Kent D C & Nadel J A *Mechanism of bronchoconstriction during inhalation* of dust J Appl Physiol 17 613 1962
19 Widdicombe J G & Nadel J A Reflex effects of lung inflation on tracheal volume J Appl Physiol 18 681 1963

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EDITORIAL

Adverse Drug Reactions—A Plea for a Realistic Attitude

Adverse drug reactions are—or should be—the concern of all physicians in surgical as well as in medical specialities The lay public—and to some extent also physicians—seem to believe that drugs entirely free from adverse reactions can be obtained This is a dream and will remain so although to talk with Hamlet a dream devoutly to be wished No drug is completely free from risk—and never will be!

A few years ago J G Waldenstrom stated in an editorial (6) that the Acta would like to collaborate in disseminating knowledge about toxic effects of drugs and the methods of recognizing them The response has not been impressive A perusal of the contents of the last ten volumes (1974–78) shows that out of close on 1000 articles only 30 i e 3% dealt directly with adverse drug reactions—and that no visible increase occurred as a result of the appeal of the Editor

The attitude towards adverse drug reactions differs widely—from that of the enthusiastic physician-therapist who believes that modern drugs do so much good that an occasional adverse reaction does not count to that of the deadly scared and tormented patient with a severe adverse reaction The American Medical Association had to abandon its registry on adverse drug reactions and recently Ingelfinger (4) said that the total number of adverse reactions per given population per year may be a figure for the record books but in itself is of hardly any pragmatic importance

It is important to realize that we have to live with adverse drug reactions—as we have learnt to live with complications to surgery The important thing is to minimize the number of such reactions as well as their negative effects when they do occur And this can be achieved by learning more about drugs about their benefits and disadvantages And knowledge in the latter aspect can be obtained only by collecting and analyzing such adverse drug reactions as have occurred and by returning such knowledge to the prescribing physicians—and also to the patients and the public at large

The problem with adverse drug reactions is to a large extent a problem of information It is as such both a difficult and a delicate problem that must be handled with great care Advantages must be balanced against disadvantages positive drug effects against risks of adverse reactions The press handles all reports on adverse drug reactions in the same way—the negative sides are grossly exaggerated and the positive effects are not mentioned at all Such negative over dramatization is harmful to our patients who get scared and do not take the drugs that are necessary for their recovery and health Even physicians get scared and refrain from using valuable drugs This type of negative and one sided information prevents a realistic and balanced attitude towards the drugs that we need in modern medicine

In Sweden all physicians have to report adverse drug reactions to a central Adverse Drug Reaction Committee which has been active since 1965 During 13 years of operation (1966–78) a total of 18000 reports have been received—the annual number has gone up from 600 to over 2200 in 1978 All incoming reports have been carefully analyzed as to the cause and-effect relation between the intake of the drug and the ensuing negative reaction The material has formed the basis for a large number of studies (≈70) that have been published—two examples are to be found in this issue

Analysis of a nation wide material of adverse drug reactions as large as that of the Swedish Committee will permit us to identify *patients at special risk* as well as tell *what adverse reactions* especially *to look for with specific drugs and what drugs are* likely to be involved when a certain negative reaction is encountered Also Swedish experiences clearly demonstrate that the results of the work with adverse drug reactions can be used to influence the drug market in the country

The number of adverse reactions rises very markedly with increasing age of the patient This is true for all occurring reactions and still more for those with a fatal outcome (cf p 452) This puts a special emphasis on the many difficulties involved in *geriatric pharmacotherapy* which although at the opposite end of the life span has at least as many special aspects and problems as neonatal and

pediatric pharmacotherapy To mention only a few elderly and old people have many simultaneous dis eases and ailments they take many drugs—but they also may have decreased drug metabolic capacity they definitely have impaired renal function and there are indications that they have increased sensitivity at the receptor level e g for dicumarol all of which changes may lead to accumulation of drugs—and adverse reactions'

Rapid changes occur in the spectrum of adverse reactions especially due to shifts among the drugs commonly prescribed This is well illustrated by Table IV in the article Drug induced Blood Dyscrasias in this issue Out of 12 drugs—or groups of drugs—responsible for drug induced cytopenias only *one* (methyldopa) remained in the same position on the list during two consecutive 5 year periods All others had appeared disappeared or changed position on the list Another example is the oral contraceptives that 10 years ago accounted for 40% of the reports to the Swedish Committee—to-day the figure is 5% Antibiotics and sulfonamides have increased from 9% in the late 1960s to 30–35% at the present time Especially the large number of sulfonamide induced deaths is disturbing The sulfonamides have although under constant development been on the market for more than 40 years This in itself is reason enough to re evaluate them and their position in modern harmacotherapy a task that becomes even more perative when they are found to cause a consid rable number of adverse reactions and even deaths

Sulfonamides to-day are used mainly on two indi cations urinary tract infections—often in combina tion with trimetoprim—and ulcerative colitis In teresting studies in progress indicate that it may be possible in both conditions to proceed without the sulfonamide component Danish studies have indi cated that trimetoprim alone might be as effective as the trimetoprin–sulfonamide combination in treatment of urinary tract infections (5) and studies in Oxford that the active moiety of sulphasalazine (Salazopyrin) may well be 5 aminosalicylic acid (1) Perhaps it is time for the sulfonamides to retire from the scene?

The results from the Swedish Adverse Drug Reaction Committee also bear witness to the useful ness of the work—definite changes on the Swedish drug market have been brought about' The Com mittee warned against the indiscriminate use of chloramphenicol—sales figures dropped and no case of chloramphenicol induced aplastic anemia has been seen in Sweden during the last 8 years Re peated warnings against dipyrone (noramidopyrine) first led to a marked and abrupt decrease in sales figures later to its disappearance from the market The Committee has been active in banning oral contraceptives with high estrogen content and late ly brought about the disappearance of thenalidin and phenformin

International cooperation is of great importance also in the field of adverse drug reactions One example of such efforts is the WHO International Drug Monitoring Centre since 1978 located in Uppsala Sweden that now receives data on adverse drug reactions from 23 countries all over the world It will be interesting to see what the Centre can accomplish in its new location International differ ences may be expected because of variations be tween systems of medical care medical education and drug usage Such differences have for exam ple explained a large part of the greatly varied occurrence of drug induced aplastic anemia be tween the Far East and the western world (3)—such aplasia is at least 4–5 times as common in the east Also pharmacogenetics are of importance—the proportion of people with e g low content of hepatic acetyl transferase or red cell glucose 6-phosphate dehydrogenase differs widely between populations

Let this suffice to demonstrate that work with adverse drug reactions is of importance and that we may learn much from it And we do need more knowledge about the drugs we use about their posi tive as well as negative effects to increase the standards of our pharmacotherapy

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REFERENCES

1 Azad Khan A K Piris J & Truelove S C An experiment to determine the active therapeutic moiety of Sulphasalazine Lancet 2 892 1977
2 Bergman U Boman G & Wiholm B E Epidemiology of adverse drug reactions to phenformin and metformin Br Med J 2 464 1978
3 Bottiger L E Epidemiology and etiology of aplastic anemia. International Symposium on Aplastic Anemia Schloss Reisenburg 1978 In press 1979
4 Ingelfinger F J Counting adverse drug reactions that count N Engl J Med 294 1003 1976
5 Vejlsgaard R Personal communication 1979
6 Waldenstrom J G Toxicology in clinical medicine (editorial) Acta Med Scand 199 378 1976

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Fatal Reactions to Drugs

A 10 Year Material from the Swedish Adverse Drug Reaction Committee

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ABSTRACT Drug induced deaths ($n=274$) in Sweden during a 10-year period have been analyzed The incidence has been remarkably constant with 25–30 reported cases per year There is a marked increase with age in the incidence of fatal reactions, more so than for all drug reactions Women consume more drugs than men and get more reactions, but not more fatal reactions Anti inflammatory drugs (antibiotics and sulfonamides) are responsible for 21% of the fatal reactions followed by oral antidiabetics (9%) oral contraceptives (9%) and antiphlogistic drugs (8%) The blood and the bone marrow are the most susceptible organs responsible for 40% of the fatal reactions, followed by thromboembolism (10%) and hepatocellular damage (9%) It is important to note that rapid changes have occurred with regard to responsible drugs as well as to the types of adverse reactions encountered

Key words deaths fatal reactions drugs drug induced anti inflammatory drugs blood bone marrow

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Deaths due to drug treatment have recently been discussed in an editorial in the British Medical Journal (8) The conclusion which we believe correct was that the picture is less serious than has been alleged However it is always necessary to be vigilant and to do everything possible to avoid even a single unnecessary death And to do this we need more knowledge of drug action and reaction i e what good and what harm our drugs do And to learn more about adverse drug reactions when to expect them how to recognize them and how to avoid them there is only one way—to collect evaluate and analyze reports of such reactions In this context it is especially important not to shrink back from the most severe ones those with fatal outcome

In Sweden a nation wide system for reporting adverse drug reactions has been functioning since late in 1965 Thus it is now possible to analyze and report the experiences from the first 10 years This is an analysis of the total material of fatal drug reactions with regard to the total incidence of such reactions as well as to drugs involved and the types of adverse reactions encountered

In Sweden all adverse drug reactions should be reported to a central Adverse Drug Reaction Committee The regulations especially stress the importance of reporting the following types of adverse reactions viz those that lead to death those that are severe and those that are unexpected and/or new Further it is stated that every physician is free—and in fact encouraged—to report all adverse reactions regardless of whether they fall into any of the mentioned categories or not

MATERIAL

All reports to the Swedish Committee are scrutinized by a medical officer who if necessary asks for additional information This is always done for the fatal—and for the more severe—cases where the complete medical record is generally obtained The medical officer performs a preliminary cause and-effect classification which is further discussed by a working party (three medical officers) of the Adverse Drug Reaction Committee and is finally confirmed by the Committee itself The latter consists of 11 members all physicians who represent pharmacology clinical pharmacology and major clinical specialities Only those reports classified as "causal relationship probable" or "causal relationship not excluded" have been accepted for the present study It should be mentioned that there is in fact little difference between the two classifications and that the causal relationship in both may be regarded as comparatively high

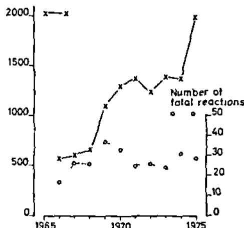


Fig 1 Total number of adverse drug reactions reported and number of fatal reactions in Sweden 1965–75

RESULTS

During the 10 years 1966–75 a total of 11 596 adverse drug reactions were reported The annual number of reports has increased gradually (Fig 1), from approximately 600 to 1 600 per year

During the same period *274 fatal drug reactions* were reported In contrast to the total number of adverse drug reactions the number of fatal cases remained remarkably constant through the with 25–30 cases annually (Fig 1)

The age- and sex related incidence of fatal reactions is shown in Fig 2 Women predominate and the overall women/men ratio is 1 5/1 0 The same ratio is found among all reports Thus women do get more adverse reactions than men but the risk of getting fatal reactions is the same for both sexes The number of fatal reactions increases very rapidly in the higher age groups even more than corresponds to the overall increase in drug reactions with age

The drugs causing fatal reactions have been listed in Table I There is a dominance of anti inflammatory drugs antibiotics and sulfonamides which together constitute 21% of the total material followed by oral antidiabetics and contraceptives antiphlogistics anesthetics and psychotropic drugs These six categories make up almost 2/3 of the total material

The type of fatal reaction is given in Table II Hematologic reactions bone marrow damage of various types, predominate and were the cause of death in 40% of the patients Thromboembolic disease and hepatocellular damage were the next most important causes of death 10 and 9% respectively

Sixty three per cent of the patients had used one or more drugs besides the one believed responsible for their death 117 patients (43%) had used 1-3 other drugs 56 patients (20%) 4 or more simultaneous drugs

DISCUSSION

To-day many countries, e g the UK Denmark New Zealand and Sweden have nation wide systems for collecting and working up reports on adverse drug reactions In other countries the work is centered around local projects in one or many hospitals—the best known example being the Boston Cooperative Drug Surveillance Program This difference as well as the wording of the regulations for reporting and the observance of them explains many of the varying results in adverse drug reaction studies The results are influenced also by the type of hospitals studied and the size of the patient materials It is important to keep this in mind when discussing adverse reactions to drugs and their importance—in medical care and in society

Comparatively few studies exist of deaths from drugs Shapiro et al (13) from the Boston group published in 1971 a study showing that 0 44% of hospital admissions got fatal reactions from therapeutic drugs Extrapolation from the rather small material led to apparently unreasonable figures for the total number of drug induced deaths in the USA

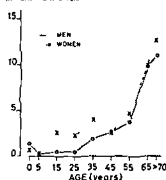


Fig 2 Incidence of fatal drug reactions in Sweden—deaths per 10^5 inhabitants and year

Table I *Drugs responsible for fatal adverse drug reactions*

Type of drug	No of pats n			%
Anti infectious drugs				
Antibiotics				
Penicillins	8			
Chloramphenicol	7			
Nitrofurantoin	7	35		
Tuberculostatics	6			
Miscellaneous	7			
Sulfonamides				
Sulfapral® (sulfamethizole + sulfamethoxipyridazine)	11			
Sulfadon® (sulfa merazine + sulfa proxyline)	4		59	21
Salazopyrin® (azulfidine)	2	24		
Trimethoprim–sulfamethoxazole	2			
Miscellaneous	5			
Antidiabetics				
Phenformin	22		27	9
Others	5			
Contraceptives oral			25	9
Antiphlogistics				
Indomethacin	8			
Oxyphenbutazone	8		23	8
Phenylbutazone	6			
Gold	1			
Anesthetics				
Halothane	17		22	7
Others	5			
Psychotropic drugs				
Phentiazines	14			
Tricyclic antidepressants	3		20	7
Others	3			
Anticoagulants				
Streptokinase	11			
Dikoumarol	5		19	6
Heparin	3			
Cytostatics			17	6
Analgesics—only dipyrone			16	5
Drugs for circulatory diseases			12	4
Drugs for pulmonary diseases			7	2
Roentgen contrast media			7	2
Thyreostatic drugs			6	2
Antiepileptics			5	2
Miscellaneous			30	10
Total no of drugs			295	100
More than one responsible drug per patient			−21	
Total no of pats			274	

* For 15 patients two and for 3 patients three drugs have been listed as responsible

Table II *Type of fatal drug reaction*
Only one reaction listed per patient

Type of reaction	No of pats n		%
Hematologic			
Agranulocytosis	70		
Aplastic anemia	28	109	40
Hemolytic anemia	6		
Thrombocytopenia	5		
Thromboembolism		28	10
Hepatocellular damage		23	9
Anaphylactic reaction		22	8
Lactic acidosis		21	8
Bleeding complications		16	6
Circulatory collapse		12	4
Toxicodermia		11	4
Interstitial pneumonitis		4	1
Miscellaneous		28	10
Total		274	100

and started a heated discussion In 1977 the Boston group reappeared with a new analysis (12) of a larger material from seven countries with a much lower mortality—0 09% In the meantime another American report (7) had given an intermediate figure—0 22% Commenting upon the debate of drug induced deaths Ingelfinger (10) pointed out that the total number of adverse reactions per given population per year may be a figure for the record books but in itself is of hardly any pragmatic importance

Our analysis provides such data but more important information on the type of fatal adverse reactions that one might expect and on the drugs that are likely to cause them The value of such studies increases when similar reports are published from other quarters and when the results are in accord A comparison between lists of drugs causing fatal reactions in the UK (9) and in Sweden (4) shows great similarities and important differences This is demonstrated in Table III which also introduces a time aspect in order to demonstrate the rapid changes that occur with time The similarities are clearly visible and need no comment The two important differences concern isoprenaline and anti infectious preparations the former high on the British list the latter notably absent The high number of isoprenaline induced deaths in the UK most likely is accounted for by the high prevalence of obstructive pulmonary disease in Great Britain The absence of antibiotics and sulfonamides from

Table III *Ranking list for drugs found to cause fatal adverse reactions in the UK and Sweden*

British material adjusted after Girdwood (9) Swedish material from two 5 year periods separately and combined to give a 10-year period

UK 1963–73	Sweden 1966–70	Sweden 1971–75	Sweden 1966–75
Antiphlogistics	Antibiotics	Antibiotics	Antibiotics
Oral contraceptives	Oral contraceptives	Oral contraceptives	Oral antidiabetics
Psychotropic drugs	Psychotropic drugs	Anesthetics	Oral contraceptives
Corticosteroids	Sulfonamides	Anticoagulants	Antiphlogistics
Isoprenaline	Anesthetics	Cytostatics	Anesthetics
Anesthetics	Antiphlogistics	Oral contraceptives	Psychotropic drugs

the British list however cannot be as easily explained

The total number of reports of adverse drug reactions in Sweden has increased gradually (Fig 1) Whether this represents an increase in the number of occurring reactions or only of reports is impossible to tell The regulations for reporting were changed in 1974 making it easier to know what to report This probably contributed to some of the recent increase Even if more and more highly specific and active drugs are being used there is however nothing to indicate that the number of adverse drug reactions should have increased rapidly Therefore most of the increase is probably due to better reporting In fact it was found in a recent ly (4) that the number of reports of adverse drug per active physician and year has gone up 0 06 in 1966 to 0 12 in 1977 i e doubled The same analysis also showed that the reporting frequency was very similar all over the country with a constant ratio between the size of the population and the number of reports from the various counties

Regardless of whether the increase of reported reactions is real or due to better reporting it is interesting to note that the number of fatal reactions remains remarkably constant during the whole 10-year period with 25–30 cases annually (Fig 1) This could indicate that all through the period all or at least most of the more severe reactions including the fatal ones were in fact reported The overall reporting frequency in Sweden has been approximately 30% (5) but indications have always pointed to the fact that the frequency with which severe or fatal reactions were reported was much higher

It is very seldom possible to *prove* that a given drug has caused a negative reaction This is especially true of the more severe reactions in which the evaluation generally has to be based on statistical and indirect evidence The causal relationship between drug and reaction in this material may however be regarded as high A careful work up is done in every case with the total medical record at hand and the evidence analyzed in several steps and by separate groups of physicians clinicians as well as pharmacologists

There are more women than men among drug induced deaths (60%) However this overrepresentation is the same as among all adverse drug reactions and corresponds well with a larger drug consumption among women Of the consumers of therapeutic drugs 57% are women with even higher figures for special drug groups such as e g analgetics (60%) (2) Thus on the whole no different susceptibility to drugs seems to exist between the sexes On the other hand there are special drugs used solely by women e g oral contraceptives which are responsible for the marked increase in fatal reactions among women 15–44 years of age (Fig 2)

Age on the other hand predisposes for more severe drug reactions For both sexes there is a marked increase in incidence after the age of 50 (Fig 2) This increase which can be noted also for adverse drug reactions on the whole has many explanations Elderly people have more illnesses than younger and take more drugs renal filtration is decreased and excretion of many drugs therefore diminished drug metabolism may be impaired (11) and susceptibility to drugs may be changed (14) It is important to develop and spread knowledge of the many problems of *geriatric pharmacotherapy* It has at least as many specific traits and problems as has neonatal or pediatric pharmacology two fields of interest that have attracted much attention—and accordingly developed rapidly

It is of special importance to keep the number of simultaneously given drugs as low as possible again especially in the elderly Many of the patients

included in this report had taken many drugs other than that believed responsible for their death—20% of the patients no less than 4 other drugs or more (up to 14!)

Drugs against infections (antibiotics and sulfonamides 21% of fatal cases) predominate among drugs causing deaths The total number of reports for antibiotics and sulfonamides in 1968 made up 15% of all reports In 1972 the figure had risen to 30% and has remained unchanged until now Thus a major part of all reports of drug induced deaths concern anti infectious drugs Definite changes have however occurred within the group during the last 10 years From the beginning *chloramphenicol* and various penicillins were most often implicated During the latter half of the observation period only one case each is caused by those drugs where as *nitrofurantoin* has risen as the most often implicated antibiotic A detailed report of nitrofurantoin induced pulmonary lesions has been published elsewhere (15) as has a discussion of adverse reactions during treatment of urinary tract infections (6)

The large number of sulfonamide induced deaths is disquieting Sulfonamides have although under constant development been in the market for more than 40 years This in itself is reason enough to re-evaluate them and their position in modern pharmacotherapy This becomes even more important when they are found to cause a considerable number of adverse reactions and deaths

Phenformin appears high on the list (Table I) a finding that has now led to withdrawal of the drug from the market in Sweden (1) *Anticoagulants* also take a conspicuously high position to a large extent due to an increased use of streptokinase with bleeding complications as an undesired effect

The deaths reported are mainly due to affections of the blood and bone marrow (40%) followed by liver damage (9%) and skin toxicity (4%) (Table II) Many fatal reactions are not organ bound but general in nature e g thromboembolism (10%) anaphylaxis (8%) and bleeding complications (6%)

The blood/bone marrow damage most often encountered is agranulocytosis (70 cases) and aplastic anemia (28 cases) There are however indications that the number of cases with fatal bone marrow damage is decreasing—67 fatal reactions were noted during 1966–70 but only 42 during 1971–75 This is due mainly to the disappearance of cases induced by chloramphenicol and dipyrone Other changes during the 10-year period are a *decrease* in the number of fatal cases of thromboembolism and an *increase* in lactic acidosis and liver damage Drug induced thromboembolism is almost solely due to oral contraceptives and the diminished incidence probably can be explained by a shift to preparations with a low estrogen content Studies are in progress to see whether this assumption is correct

It has already been demonstrated (3) that rapid changes can be noted with regard to the drugs used as well as to the types of adverse reactions encountered Many changes occur spontaneously New and serious reactions appear such as chronic interstitial pneumonitis after nitrofurantoin—four fatal cases were noted during the last 5 year period—or lactic acidosis after phenformin Other changes can be attributed to the activities of the Adverse Drug Reaction Committee Even if some of the results have already been discussed it is worth mentioning that the Committee has warned against the indiscriminate use of chloramphenicol—with a resulting decrease in sales figures and a virtual disappearance of chloramphenicol induced aplasia—and brought about the disappearance of dipyrone and accordingly of dipyrone induced agranulocytosis It has been active in banning oral contraceptives with a high estrogen content and phenformin These few examples clearly demonstrate the value of active work with adverse drug reactions

REFERENCES

1 Bergman U Boman G & Wiholm B E Epi demiology of adverse drug reactions to phenformin and metformin Br Med J 2 464 1978
2 Bergstrom I Carmstad A Elwin C E Heedman P A Kallstrom B Moell B Swarén U Wester holm B & Wiman F Lakemedelsregistrering i Jamtlands lan Erfarenheter från en förstudie 1968 (The registration of drug consumption experience gained from a preliminary study in 1968 in the county of Jamtland) Lakartidningen (Suppl) 1 38 1970
3 Bottiger L E Adverse drug reactions An analysis of 310 consecutive reports to the Swedish Adverse Drug Reaction Committee J Clin Pharmacol 13 373 1973
4 Bottiger L E Hast R & Holmberg L Drug induced cytopenias in Sweden Lakartidningen 76 860 1979
5 Bottiger L E & Westerholm B Adverse drug reactions during treatment of urinary tract infections Br Med J 3 339 1973
6 — Vem anmaler lakemedelsbiverkningar? (Who re

ports adverse reactions to drugs?) Eur J Clin Pharmacol 11 439 1977
7 Caranasos G J Franklin E M Stewart R B & Cluff L E Drug associated deaths of medical in patients Arch Intern Med 136 872 1976
8 Editorial Deaths due to drug treatment Br Med J 1 1492 1977
9 Girdwood R H Deaths after taking medicaments Br Med J 1 501 1974
10 Ingelfinger F J Counting adverse drug reactions that count N Engl J Med 294 1003 1976
11 O Malley K Crooks J Duke E & Stevenson I H Effect of age and sex on human drug metabolism Br Med J 3 607 1971
12 Porter J & Jick H Drug related deaths of medical inpatients JAMA 237 879 1977
13 Shapiro S Slone D Lewis G P & Jick H Fatal drug reactions among medical inpatients JAMA 216 467 1971
14 Shepherd A M M Wilson N & Stevenson I H Warfarin sensitivity in the elderly In Drugs and the elderly (ed J Crooks & I H Stevenson) MacMillan New York In press 1979
15 Strandberg I Wengle B & Fagrell B Chronic interstitial pneumonitis with fibrosis during long term treatment with nitrofurantoin Acta Med Scand 196 483 1974

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Drug-Induced Blood Dyscrasias

A Ten Year Material from the Swedish Adverse Drug Reaction Committee

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ABSTRACT Drug induced blood dyscrasias (agranulocytosis, aplastic anemia, hemolytic anemia and thrombocytopenia) in Sweden during a 10 year period (1966–75) have been analyzed The overall occurrence is remarkably constant, although marked changes have occurred with regard to offending drugs Drug induced thrombocytopenia and agranulocytosis are about twice as common as hemolytic anemia, which in turn is twice as common as aplastic anemia There is a marked increase with age in the incidence of all drug induced cytopenias Women predominate and make up close to 70% of the material With regard to responsible drugs, the most remarkable finding is the high frequency with which sulfonamides appear as responsible for all types of drug induced cytopenia

Key words cytopenia agranulocytosis aplastic anemia hemolytic anemia thrombocytopenia drug induced sulfonamides

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Blood dyscrasias feature prominently among adverse drug reactions In the material of the Swedish Adverse Drug Reaction Committee (3) blood disorders make up 10% of the total number of reports—only cutaneous reactions (25%) and liver damage (12%) are registered more frequently The importance of blood dyscrasias is even greater among the reported reactions with a fatal outcome—in two consecutive studies (4 5) damage to blood and bone marrow accounts for 48 and 41% respectively

In all work with adverse drug reactions it is important *firstly* carefully to analyze the cause and-effect relationship between the taking of a drug and the ensuing negative reaction *secondly* regularly to analyze and work up the collected material and *thirdly* to publish the results This is the only way to spread knowledge about reactions a knowledge that makes it possible to minimize the number of adverse drug reactions and their negative effects

This is an analysis of the reported drug induced cytopenias (agranulocytosis aplastic anemia hemolytic anemia and thrombocytopenia) reported to the Swedish Adverse Drug Reaction Committee during the first 10 years of its work (1966–75) The results demonstrate a remarkably constant occurrence of such reactions although marked changes have taken place with regard to the offending drugs

MATERIAL AND METHODS

A nation wide system for reporting adverse drug reactions has been functioning in Sweden since late in 1965 All reports to the Swedish Adverse Drug Reaction Committee are scrutinized by a medical officer who if necessary asks for additional information This is always done in the fatal and more severe cases where the complete medical record is generally obtained The medical officer performs a preliminary cause and-effect classification which is further discussed by a working party (three medical officers) of the Committee and is finally confirmed by the Committee itself The latter consists of 11 members all physicians who represent pharmacology clinical pharmacology and major clinical specialties Only those reports classified as Causal relationship probable or Causal relationship not excluded have been subjected to the present study It should be mentioned that these two categories which make up approximately 80% of the reports both carry a high degree of causal relationship and there is in fact little difference between them

RESULTS

A total of close to 11 600 reports of adverse drug reactions were received by the Committee during the 10-year period 1966–75 The annual number of reports has increased gradually and in the mid 70s was approximately 1 600 per year (Fig 1) The

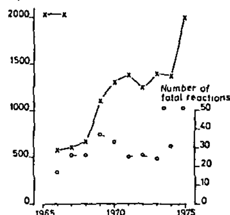


Fig 1 Total number of adverse drug reactions reported and number of fatal reactions in Sweden 1965–75

number of drug induced deaths has remained remarkably constant 25–30 cases per year (Fig 1)

During the same time *592 cases of drug induced cytopenias* were reported (Table I) Drug induced thrombocytopenia is slightly more common than agranulocytosis and both are much more common than *drug induced hemolytic and aplastic anemia* respectively Another ranking order between the cytopenias emerges if the analysis is confined to the fatal cases (Table I) Most deaths were due to agranulocytosis (n =63) and aplastic anemia (n =26) ereas drug induced hemolytic anemia and thrombocytopenia carry a much better prognosis with only a few fatal cases in each category

The number of drug induced cytopenias has *remained remarkably constant during the 10-year* period The yearly incidence calculated for two consecutive 5 year periods (Table II) shows a possible slight increase in the number of drug induced hemolytic anemias but virtually no changes in the other cytopenias

There is a marked increase with age in the incidence of all drug induced cytopenias with the exception of aplastic anemia which shows only a slight increase (Fig 2) Women predominate among drug induced cytopenias (Table I)—69% of the whole material The greatest predominance is found for drug induced hemolytic anemia where autoantibodies are known to be the pathogenetic factor and the lowest for aplastic anemia where a toxic mechanism is the most likely

The three most often reported drugs or drug

Table I *Total number of various drug induced cytopenias 1966–75 fatal cases and sex ratio*

	Total no of pats	Deaths n	Deaths % of total reports	Women (%)
Agranulocytosis	199	63	32	70
Aplastic anemia	51	26	51	61
Hemolytic anemia	109	4	4	76
Thrombocytopenia	233	6	3	67
Total	592	99	17	69

groups causing the respective cytopenias are given in Table III These top three are responsible for 51–84% (mean 65) of all drug induced cytopenias and for 40–75% (mean 42) of all fatal cytopenias It is important to note the high frequency with which sulfonamides appear on the list of offending drugs (see below)

DISCUSSION

The observed increase in the number of reports of adverse drug reactions received by the Swedish Committee (Fig 1) probably does not represent a true increase in the actual number of reactions but depends largely on better reporting Regardless of this there is however reason to believe that the more severe cases including the fatal reactions have been reported all the time much more frequently than the average of 30% which several independent studies have found for all reports of *adverse reactions in Sweden (8)*

The number of drug induced cytopenias has remained remarkably constant There seems to be a small increase in the number of drug induced cases of hemolytic anemia but the other three cytopenias occur with almost the same frequency during the

Table II *Yearly incidence of drug induced cytopenias calculated per million inhabitants for two consecutive 5 year periods*

	1966–70	1971–75
Agranulocytosis	2 5	2 6
Aplastic anemia	0 7	0 6
Hemolytic anemia	1 1	1 6
Thrombocytopenia	3 1	2 7

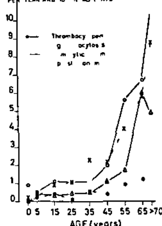


Fig 2 Average distribution by cases per 10^5 inhabitants per year and by age of drug induced cytopenias in Sweden in the 10-year period 1966–75

two 5 year periods investigated (Table II) This is remarkable considering that marked changes have occurred with regard to responsible drugs (vide infra) This could be taken as an indication that individual susceptibility plays an important role more important perhaps than the offending drugs In other words at least at a low degree of exposure to toxic or sensitizing drugs a number of sensitive individuals will develop negative reactions to a variety of drugs

There are marked differences between the four drug induced cytopenias with regard to incidence prognosis age and sex distribution and the offending drugs Drug induced thrombocytopenia and agranulocytosis are the most common varieties each approximately twice as common as drug induced hemolytic anemia which in turn is twice as common as aplastic anemia (Table I) Drug induced agranulocytosis hemolytic anemia and thrombocytopenia show a marked increase with age which is not the case for aplastic anemia Women predominate (Table I) in all groups accounting on average for 70% of the material Women do consume more drugs than men—55–60% of consumers of therapeutic drugs are women (2)—but the female predominance among the drug induced cytopenias (as in other materials of adverse drug reactions) is higher and indicates that women not only consume more drugs than men but get relatively more damage from them

The most often implicated drugs—or groups of

Table III *The three most commonly reported drug groups/drugs causing the respective cytopenias*
Total no (and no of deaths) in absolute figures

	Total no	Deaths
Agranulocytosis		
Sulfonamides	46	8
Dipyrone (noramidopyrine)	38	12
Thyreostatics	29	5
	113	25
% of total reports	57	40
Aplastic anemia		
Sulfonamides	11	2
Oxyphenbutazone	10	5
Chloramphenicol	5	4
	26	11
% of total reports	51	42
Hemolytic anemia		
Methyldopa	69	3
Sulfonamides	14	–
Dapsone (Avlosulfon)	9	–
	92	3
% of total reports	84	75
Thrombocytopenia		
Diuretics	81	2
Quinine quinidine	43	–
Sulfonamides	27	1
	151	3
% of total reports	65	50

drugs—are given in Tables III and IV Although some drugs appear as the cause of more than one type of cytopenia the lists for the various types differ on the whole

Drug induced agranulocytosis is caused in order of falling frequency by sulfonamides dipyrone (novalgin noramidopyrine) and thyreostatic drugs The sulfonamides will be discussed later (vide infra) Dipyrone (noramidopyrine) is a derivative of —and chemically closely related to—amidopyrine a drug well known to cause agranulocytosis in fact one of the first drugs for which the relationship between drug and agranulocytosis could be established Dipyrone did cause a large number of cases of agranulocytosis in Sweden (8) even fatal ones (n = 12) until it was taken off the market in 1974 The

Table IV *Most commonly reported drugs/groups of drugs as the cause of the various cytopenias during two consecutive 5 year periods*

	1966–70	1971–75
Agranulocytosis	Dipyrone (noramidopyrine)	Sulfonamides
	Thyreostatics	Thenalidine
	Sulfonamides	Thyreostatics
Aplastic anemia	Oxyphenbutazone	Sulfonamides
	Chloramphenicol	Cytostatics
	Phenylbutazone	Acetazolamide
Hemolytic anemia	Methyldopa	Methyldopa
	Sulfonamides	Avlosulfon
	Avlosulfon	Sulfonamides
Thrombocytopenia	Diuretics	Sulfonamides
	Quinine/quinidine	Diuretics
	Phenylbutazone	Quinine/quinidine

frequency with which thyreostatic drugs cause agranulocytosis has remained constant with a relation between the three commonly used drugs (carbimazole tiamazole and propylthiouracil) that corresponds to the sales figures i e the risk seems to be similar for them all

The shift between causative drugs has been especially remarkable for *drug induced aplastic anemia* Chloramphenicol was for many years—and all over the world—the drug that most often elicited aplasia In a previous study (7) it was found that in Sweden yphenbutazone and phenylbutazone had re laced chloramphenicol and taken the leading position Some years later this finding was confirmed from Australia (9) and in the WHO reports from the monitoring center in Geneva (11) And now even the antiphlogistics have been overtaken by sulfonamides which top the list It should be pointed out that this is a matter of a real and absolute increase in the number of reported cases of sulfonamide induced aplasia not just an unmasking because of a decrease in other drugs The total figures for the 10-year period are somewhat misleading, inasmuch as no case of chloramphenicol induced or oxyphenbutazone/phenylbutazone induced aplasia was found during the last 5 years

Drug induced hemolytic anemia is caused by methyldopa in the vast majority of cases (75%) The number of patients with methyldopa induced hemolytic anemia has more than doubled—from 21 cases in 1966–70 to 48 cases in 1971–75 It should be mentioned that these are all overt and manifest cases of severe hemolysis—no cases have been included solely because of a positive Coombs test A more detailed study has been published previously (10) The tendency in Sweden seems to be to use methyldopa less often which seems reasonable even from the light of adverse effects

Drug induced thrombocytopenia is caused mainly by oral diuretics with furosemide as the leading variety followed by chlorthalidone and various thiazides in proportions well compatible with their sales figures A previous study (6) found no differences among the modern diuretics in their capacity to induce thrombocytopenia Another classic in the field of drug induced disease quinine and more recently also quinidine remains high on the list of drugs causing thrombocytopenia In third place come sulfonamides with increasing frequency during the 10-year period

The marked overall changes in the spectrum of drugs inducing cytopenias are demonstrated in Table IV—only one drug (methyldopa) remains in the same position during the two 5 year periods analyzed All others have appeared disappeared or altered their position on the list

The most striking fact with regard to offending drugs is the dominance of sulfonamides, which appear as responsible for a considerable number of cases of all the four types of cytopenia—as the leading drug in two (agranulocytosis and aplastic anemia) as number two (hemolytic anemia) and three (thrombocytopenia) in the others Moreover one is faced with an absolute increase in the number of sulfonamide induced cases in all categories except hemolytic anemia The denomination sulfonamides here includes only three preparations viz *Sulfapral®* (a two layer combination of sulfamethizole and sulfamethoxypyridazine) *Salazopyrin®* (azulfidine) and *trimethoprim sulfamethoxazole* The latter is included among the sulfonamides since it has been demonstrated that the picture of adverse reactions closely imitates that of the sulfonamides (12) and that the folic acid antagonist, trimethoprim, contrary to initial fears evidently does not cause any significant adverse reactions There is however nothing to indicate that these three sulfonamides are more harmful than others The large number of adverse reactions encountered reflects their dominance on the sulfonamide market in Sweden But the results should perhaps lead to a discussion of the value of the sulfonamides which have now been used for almost 40 years

One may well ask if this type of analysis and

work up of adverse drug reactions is worthwhile Swedish experience shows that they are of definite value The work of the Adverse Drug Reaction Committee including analyses such as the present one has actively contributed to changes in the drug market in Sweden Even if the big changes are comparatively few, there are good reasons to believe that they have saved lives The Committee warned against the indiscriminate use of chloramphenicol sales figures went down (8) and no case of chloramphenicol induced aplastic anemia has been registered during the last 8 years Repeated warnings against the use of dipyrone first led to diminishing sales figures (8) later to its total disappearance Recently thenalidine an antihistamine that caused a number of cases of agranulocytosis in the same way has been taken off the market In fields other than blood disorders it is worth mentioning that the Committee was active in banning the oral contraceptives with a high estrogen content and that recently it has brought about the disappearance of phenformin a drug causing a number of fatal cases of lactic acidosis (1)

REFERENCES

1 Bergman U Boman G & Wiholm B E Epidemiology of adverse reactions to phenformin and metformin Br Med J 2 464 1978

2 Bergstrom I Carmstad A Elwin C E Heedman P A Kallstrom B Moell B Swaren U Westerholm B & Wiman F Lakemedelsregistrering i Jamtlands lan Erfarenheter från en förstudie 1968 (The registration of drug consumption experience gained from a preliminary study in 1968 in the county of Jamtland) Lakartidningen (Suppl) 1 38 1970

3 Bottiger L E Adverse drug reactions An analysis of 310 consecutive reports to the Swedish Adverse Drug Reaction Committee J Clin Pharmacol 13 373 1973

4 Bottiger L E Furhoff A K & Holmberg L Lakemedelsbiverkningar i Sverige I Biverkningar med dodlig utgång Lakartidningen 74 1182 1977

5 Bottiger L E Nordlander M Strandberg I & Westerholm B Deaths from drugs J Clin Pharmacol 14 401 1974

6 Bottiger L E & Westerholm B Thrombocytopenia II Drug induced thrombocytopenia. Acta Med Scand 191 541 1972

7 — Aplastic anaemia II Drug induced aplastic anaemia Acta Med Scand 192 319 1972

8 — Drug induced blood dyscrasias in Sweden Br Med J 3 339 1973

9 Firkin F C Personal communication 1976

10 Furhoff A K Adverse reactions with methyldopa —A decade s reports Acta Med Scand 203 425 1978

11 de Gruchy G C Drug induced blood disorders p 39 Blackwell Scientific Publications Oxford London Edinburgh and Melbourne 1975

12 Nilsson E Biverkningar av trimetoprim sulfametoxazol ar framst sulfabiverkningar (Adverse reactions from trimetoprim sulfametoxazol are mainly of the sulfonamide type) Ronden 3 46 1975

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A Case of Merbromin (Mercurochrome®) Intoxication Possibly Resulting in Aplastic Anemia

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ABSTRACT A patient is described who appeared to be suffering from mercury intoxication caused by local application of merbromin to an operation wound and who developed aplastic anemia, which we ascribed to merbromin

Key words merbromin intoxication aplastic anemia
Acta Med Scand 205 463 1979

Although merbromin (Mercurochrome®) has been widely used since its introduction the possibility of absorption is not always appreciated Merbromin intoxication from local application to omphaloceles has been described in babies (8 10) Because intoxication in adult patients is not mentioned in the literature we thought it of interest to report this case The subsequent development of aplastic anemia in this patient can probably be ascribed to merbromin

CASE REPORT

A 59 year-old woman was admitted in 1976 for reconstructive surgery of an esophageal stricture due to a diaphragmatic hernia She had suffered in the past from seronegative rheumatoid arthritis for which no treatment had been given since 1972 gold compounds had never been administered

Laboratory findings on admission (Fig 1) ESR (Westergren 1 h) 15 mm Hb 14 9 g/dl Hct 45% WBC 14000/mm³ platelet count 320000/mm³ blood smear normal Routine blood chemistry gave normal results

In June 1976 a thoraco-abdominal operation was performed and the strictured part of the esophagus was removed Because further reconstructive surgery had to be delayed the distal part of the esophagus was connected to the skin in the supraclavicular space and the stomach was fixed to the abdominal wall as a feeding fistula.

In the postoperative period (Fig 1) the patient developed fever a chest X ray showed a paracardial infiltration She was treated with kanamycin and cloxacillin Because the temperature rose again after an initial amelioration gentamicin was given instead From the first post operative day merbromin in a 2% aqueous solution was applied to the surgical wounds and decubitus areas

The following drugs were administered from the immediate preoperative period until death aminophylline butylscopolamine chloralhydrate cloxacillin diazepam digoxin droperidol ethomidate fentanyl frusemide gentamicin heparin kanamycin magnesium sulfate merbromin methadone nitrous oxide pavulon pentazocine phenprocoumon phytomenadione and suxamethonium Starting on the sixth postoperative day the kidney function deteriorated (creatinine 150 μmol/l) and slight proteinuria developed On the 17th postoperative day the platelet count had fallen to 34000/mm³ and on the following day the WBC was 2100/mm³ (neutrophils 1200/mm³) After that the hematological values declined steadily

On the 20th postoperative day the reticulocyte count was only 2 / Bone marrow aspiration material showed no signs of hematopoiesis Bone marrow histology (described by J te Velde) showed many fat cells and no signs of hematopoiesis Massive infiltration with plasma cells lymphocytes and macrophages as well as edema were present the sinus walls were disrupted and edematous The picture was characteristic for aplastic anemia Because merbromin intoxication was considered the mercury content of blood and urine was determined A blood sample (on the 22nd day) contained 700 μg/l (normal 59±26 μg/l ref 4) and the urine 800 μg/l mercury (>100 μg/l indicative of enhanced exposure to mercury) On the 23rd day the patient died in therapy resistant shock

We did not use chelating agents—dimercaprol N acetyl DL penicillamine or calcium disodium-edetate—because the possibility of merbromin intoxication was only considered shortly before the patient died Furthermore it is not clear whether the merbromin molecule or a mercury ion is the toxic agent

Post mortem examination confirmed the clinical diagnosis i e sepsis and aplastic anemia The kidney showed aspecific changes which were not consistent with mercury intoxication

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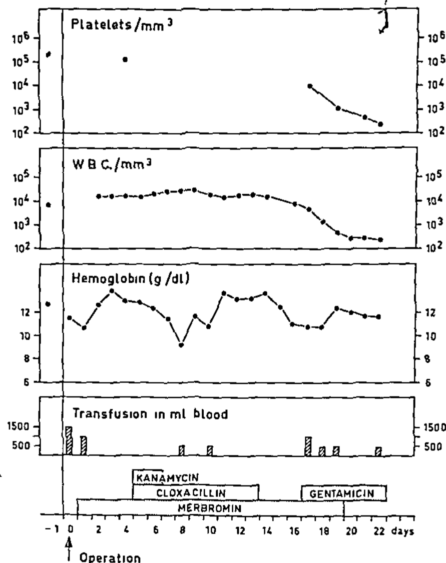


Fig 1 Course of the disease laboratory data (Values for platelets and WBC are given on a semilogarithmic scale values for Hb on a linear scale)

Toxicological analysis

Mercury determinations in the urine blood and tissues were performed by A Osinga in duplicate by cold vapour atomic absorption spectrophotometry The methods used for sample preparation were derived from the procedures described by Hatch and Ott (2) Lindstedt (5) Uthe et al (11) and Skare (9) Urine samples (2 ml) were treated with nitric and sulfuric acid and potassium permanganate Heparinized blood samples (2 ml) were oxidized with sulfuric acid and potassium permanganate Tissue samples (300 mg) were digested with concentrated sulfuric acid and after dilution with potassium permanganate Mercury vapor was released from oxidized samples by addition of hydroxylamine hydrochloride and stannous chloride Measurements were performed at 253 7 nm with a Perkin Elmer 460 Atomic Absorption Spectrophotometer equipped with a mercury hollow cathode lamp and a mercury analysis system The results are given in Table I

DISCUSSION

In our patient a tentative diagnosis of merbromin intoxication was made and was substantiated by the detection of increased mercury levels in the blood

Table I *Total mercury content (μg/g wet tissue) of three organs in our patient and the reference values given by Kitamura et al (4)*

	Patient s values	Reference values (mean±S D n=30)
Liver	1 1	0 47±0 26
Pancreas	0 7	0 083±0 048
Kidney	2 4	1 1±0 67

Table II *Data from the literature on the association of mercury and hematological complications*

Mercury compounds	Hematological observations	Mercury levels	Authors
Elemental mercury	Hb 6 8 g/dl WBC 2400/mm³ platelets 10000/mm³ hypocellular bone marrow	Urine 674 μg/24 h	Wilson (13)
	Hb 11 8 g/dl WBC 900/mm³ platelets <10000/mm³ hypocellular bone marrow	Urine 1010 μg/l	Ryne et al (7)
Intravenous elemental mercury	WBC 2100/mm³ platelets 120000/mm³ bone marrow not reported	Blood 125 μg/l urine 90 μg/24 h	Devling and Sudlow (1)
Ammonium mercury-chloride (inorganic mercurial)	Anemia leucopenia eosinophilia bone marrow not reported	Not given	Young (14)
Mercurial diuretics (organic mercury compound)	Thrombocytopenia agranulocytosis bone marrow not reported	Not given	Meyboom (6)

and urine during life and in the liver pancreas and kidney at autopsy

The minimum toxic concentrations of mercury are not clearly defined The correlation between the severity of a mercury intoxication and the mercury levels in blood or urine is poor Urinary mercury levels of 100–300 μg/l can be found in the absence of toxic symptoms (3) Kitamura et al (4) reported mercury concentrations in organs for a group of normal Japanese subjects who died due to an accident and were not known to be especially ex posed to mercury compounds These values are given in Table II together with the concentrations found in our patient

Absorption of locally applied merbromin can re sult in intoxication Schippan and Wehran (8) studied 13 babies who had been treated with merbromin on omphaloceles and found in six of them significantly increased mercury levels in the urine reaching concentrations of up to 16 mg/l Stanley Brown and Frank (10) described a newborn baby who died on the fifth day due to intoxication with merbromin which had been painted on a giant omphalocele The mercury level in the blood was 300 μg/l Neither of these publications mention hematological abnormalities

Several reports suggest that administration of mercury compounds can cause hematological tox icity (Table II) No reports concerning hema tological complications due to merbromin intoxi cation have been published

Aplastic anemia was diagnosed in our patient be cause the criteria for this disease—pancytopenia and hypocellular bone marrow—were met (12) Although a number of drugs were used merbromin is suggested as the most probable etiologic agent since none of the other drugs has been mentioned in association with aplastic anemia Other explana tions (e g rheumatoid arthritis certain infections such as hepatitis of miliary tuberculosis) were not indicated by the patient s history

REFERENCES

1 Devlin H B & Sudlow M Peripheral mercury em bolization occurring during arterial bloodsampling Br Med J 1 347 1976
2 Hatch W R & Ott W L Determination of sub-microgram quantities of mercury by atomic absorp tion spectrophotometry Anal Chem 40 2085 1968
3 Joselow M M Louria D B & Browder A A Mercurialism environmental and occupational as pects Ann Intern Med 76 119 1972
4 Kitamura S Sumino K Hayakawa K & Shibata T Mercury content in human tissues from Japan In Effects and dose-response relationships of toxic met als (ed G F Nordberg) pp 290–298 Elsevier Scientific Publishing Co Amsterdam 1976
5 Lindstedt G A rapid method for the determination of mercury in urine Analyst 95 264 1970
6 Meyboom R H B Metals In Side effects of drugs vol 8 (ed L Meyler & A Herxheimer) p 520 Excerpta Medica Amsterdam 1975
7 Ryne D R Toghill P J Tanna M K & Galan G N Marrow suppression from mercury poisoning? Br Med J 1 499 1970
8 Schippan R & Wehran H J Beitrag zur konserva tiven Nabelschnurbruch Behandlung mit Mercuro-chrom Z Kinderchir 6 319 1968
9 Skare I Microdetermination of mercury in biologi cal samples Analyst 97 148 1972
10 Stanley Brown E G & Frank J E Mercury poi

soning from application to omphalocele JAMA 216 2144 1971
11 Uthe J F Armstrong F A J & Stainton M P Mercury determination in fish samples by wet digestion and flameless atomic absorption spectrophotometry J Fisheries Research Board of Canada 27 805 1970
12 Williams D M Lynch R E & Cartwright G E Drug induced aplastic anemia Semin Hematol 10 195 1973
13 Wilson D R Mercurial poisoning and aplastic anemia. Br Med J 4 1534 1966
14 Young E Ammoniated mercury poisoning Br J Dermatol 72 449 1960

Syncope Caused by Lithium Treatment

Report on Two Cases and a Prospective Investigation of the Prevalence of Lithium Induced Sinus Node Dysfunction

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ABSTRACT Lithium salts have been widely used for several years in the treatment of manic-depressive psychosis Various side-effects of lithium salts have been described The present case reports present two patients in whom sinus node dysfunction leading to syncope was caused by lithium One of the cases showed signs of depressed sinus node function even when not on lithium, but no symptoms arose until lithium treatment was commenced The second case showed no signs of depressed sinus node function when lithium was withdrawn To study the prevalence of sinus node dysfunction in patients on lithium therapy, 97 consecutive patients on lithium were examined The examination included case history, ECG and carotid massage In two patients lithium could not be ruled out as being responsible for sinus node depression and in one patient the same was true for the atrioventricular node None of these patients had any symptoms It is concluded that lithium treatment may result in sinus node dysfunction This side-effect is however, not common Lithium treatment can obviously be instituted in all patients without a history suggesting sinus node dysfunction Patients with a history of dizziness and/or syncope should not be given lithium until thorough cardiological examination has been carried out Likewise, a cardiological examination should be performed if patients on lithium develop symptoms of this type

Key words lithium syncope sinus node dysfunction

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Lithium salts have been widely used for several years in the treatment of manic depressive psychosis (1 6) Various side effects of lithium salts have been described (5) It is well recognized that lithium may interfere with cardiac function in different ways Depression of the ST segment and/or flattening of the T wave are the most usual ECG anomalies (2) Ventricular tachyarrhythmia has been reported (9 10) Lithium has been suspected to induce diffuse interstitial myocarditis with lethal outcome (8 11)

Sinus node dysfunction caused by lithium has to our knowledge been reported in altogether four cases (3 4 12 13) Two additional cases will be presented in this report Since this type of side effect is of great practical importance an investigation was performed to study the prevalence of sinus node affection in a series of 97 patients on lithium treatment

CASE REPORTS

Case 1

This 75 year old woman who had suffered from manic depressive disease since 1940 was treated at a psychiatric hospital from 1952 to 1961 From 1962 to 1974 she was treated with chlodiazepoxide (Librium® Roche) and imipramine (Tofranil® Geigy) In 1974 she developed a state of confusion and depression After readmission to hospital she was treated with electroconvulsive therapy levopromazine (Nozinan® Leo) and thioridazine (Mallorol® Sandoz) Lithiumsulphate (Lithionit® Hassle) was instituted in a dose of 0 66 g daily She improved and was discharged from the hospital During the period 1974–76 repeated determinations of the serum levels of lithium gave values of 0 6–0 8 mmol/l

In Feb 1975 the patient suffered from dyspnoea and a chest X ray revealed slight cardiac enlargment She was given 0 25 mg of digoxin daily In March–May 1975 she consulted her district medical officer owing to dizziness She received atropine and hyoscyamine because of bradycardia In May 1975 the patient was brought to hospital because of syncope ECG showed nodal rhythm with a ventricular rate of 40–50/min Serum electrolytes were normal Digitalis intoxication was suspected and digoxin was withdrawn Her plasma digoxin level however was only 0 6 ng/l

In July 1976 the patient was readmitted because of repeated syncopal attacks On admission she was apathetic and tired and hypothyroidism was suspected This diagnosis was not confirmed by either laboratory tests (including TSH) or the subsequent clinical course Serum electrolytes were normal Lithium in serum was 0 3

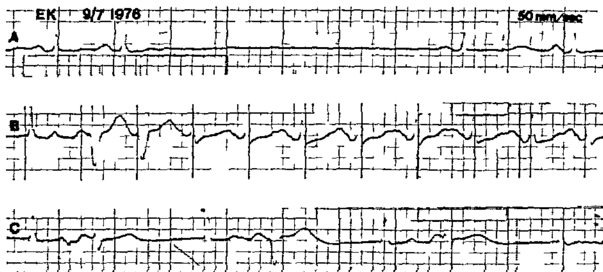


Fig 1 ECG from case 1 *A* an episode of sinus arrest with an asystole of 3 sec duration *B* sinus tachycardia (1?0/min) and two probably aberrantly conducted beats *C* nodal rythm with retrograde atrial activation and echo beats

mmol/l 48 hours after the last dose ECG revealed alternating sinus rhythm with a heart rate of 35/min sinus arrest with periods of asystole lasting 3–5 sec and slow nodal rhythm Episodes of supraventricular tachycardia ventricular premature beats and a short run of ventricular tachycardia were also noted during the continuous ECG monitoring (Fig 1)

Lithium was withdrawn owing to suspicion of lithium induced sinus node dysfunction Two days later the patient had stable sinus rhythm The ST and T wave abnormalities did not normalize however until the tenth day after lithium withdrawal (Fig 2)

Later an invasive electrophysiological investigation including a His bundle electrogram and determination of

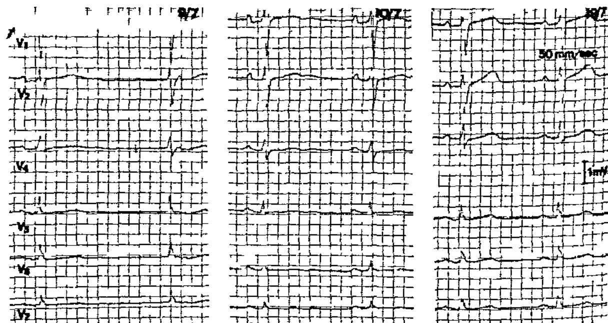


Fig 2 ECG from case 1 9/7 Sinus arrest and nodal escape 10/7 Sinus rhythm but still ST and T abnormality 19/7 Normal ECG

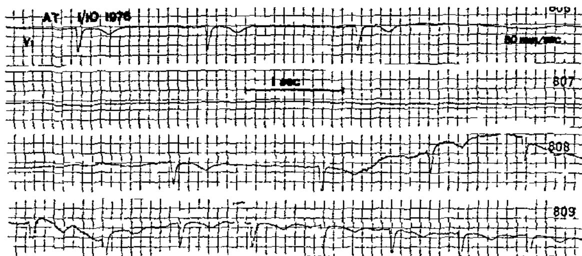


Fig 3 ECG from case 2 Sinus node dysfunction with prolonged periods of asystole

the recovery time of the sinus node disclosed a normal atrioventricular conduction system but the sinus node function turned out to be depressed The corrected sinus recovery time was 1700 ms (normal<500)

In April 1978 the patient was well without any further syncopal attacks or dizziness An ECG showed normal sinus rhythm The psychiatric disease was treated with levopromazine and thioridazine

Case 2

This 63 year-old man had had several episodes of malaria in 1948–52 He was known to have had manic-depressive psychosis since 1955 and this disease led to continuous hospital care from 1955 to 1966 and on several occasions in 1971–76 During this time the patient received electroconvulsive therapy chlorpromazine (Hibernal® Leo) and haloperidole (Haldol® Leo) In 1970 treatment with lithium sulphate was instituted (Lithionit® Hassle) and repeated checks of the serum level during 1970–76 showed values between 0 4 and 1 2 mmol/l

In 1972 the patient had an attack of unconsciousness without known cause In Sept 1976 he was admitted to hospital because of several syncopal attacks On admission the serum electrolytes were normal and the serum level of lithium was 1 1 mmol/l There was no suspicion of hypothyroidism ECG showed alternating sinus tachycardia (rate 130/min) normal sinus rhythm and pronounced sinus bradycardia (rate 20/min) There were episodes of asystole without any escape rhythm lasting about 10 sec (Fig 3) A side-effect of lithium was suspected Five days after withdrawal of lithium all arrhythmias had vanished A His bundle electrogram was normal as was the recovery time of the sinus node

Withdrawal of lithium was however followed by relapse to a psychotic state and lithium sulphate was reinstituted Two weeks later a carotid massage resulted in prolonged asystole A renewed investigation of the sinus node recovery time gave abnormal findings The corrected recovery time was 370–6400 ms Continued lithium treatment was considered necessary and the patient was therefore provided with a pacemaker In April 1978 he was quite well with no further attacks of syncope

PREVALENCE OF SINUS NODE DYSFUNCTION IN PATIENTS ON LITHIUM THERAPY

Patients and methods

An investigation has been performed to see whether sinus node dysfunction is a common phenomenon in patients on lithium treatment All patients on lithium therapy at the Psychiatric Out patient Clinic were called for examination during Jan July 1977 This included case history ordinary 12 lead ECG ECG during carotid massage and analysis of the serum level of lithium In some patients this procedure was repeated after lithium withdrawal and a couple of patients underwent invasive electrophysiological investigation The patient series consisted of 47 women and 50 men aged 24–74 years (mean+S D 48±14)

RESULTS

At the investigation the concentrations of lithium in serum were within the therapeutic range in all patients (mean±S D 0 8±0 3 mmol/l) As expected the R–R and P–Q intervals increased significantly during carotid sinus massage (Fig 4) Out of the total of 97 patients 91 had a maximum R–R interval of less than 3 sec during this procedure They were regarded as probably reacting normally Six patients had asystole exceeding 3 sec

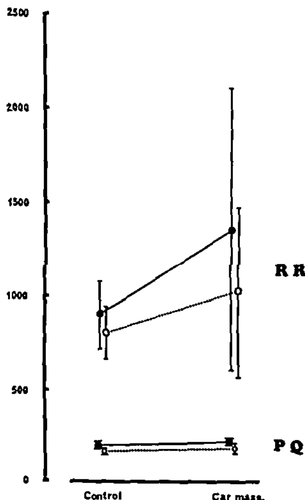


Fig 4 Effect of carotid sinus massage (*car mass*) on the R-R and P-Q intervals (mean±S E) There is a statistically significant prolongation of both intervals ($p<0\ 01$) — = Men --- = women y axis shows the interval (ms)

during carotid stimulation Pertinent data concerning these patients are presented in Table I

For different reasons, lithium was not considered responsible in three cases (nos 22 49 and 101) In two patients (nos 35 and 47) lithium could not be ruled out as being responsible for sinus node depression and in one (no 14) the same was true for the atrioventricular node None of these patients had any symptoms Since the indications for treatment were strong lithium administration was continued in all patients but one (no 14) Six months later the patients were still free from symptoms such as dizziness or syncope

DISCUSSION

The findings in our two patients presented in the case reports correspond well with the findings in four previously reported cases (3 4 12 13) Against this background a connection between lithium treatment and sinus node dysfunction is evident It should be emphasized that the serum levels of lithium have been within the accepted therapeutic range in all cases Thus, this is a side effect and not a manifestation of intoxication Lithium can induce hypothyroidism (7) and this disease may be accompanied by sinus node depression Such a mechanism has been ruled out in our two cases and does not seem probable in the cases reported previously One of our patients (case 1) had depressed sinus node function even when lithium was withdrawn This however did not lead to any symptoms until lithium therapy was resumed Case 2 in accordance with the previously reported cases did

Table I *Patients in whom the maximum R-R interval exceeded 3 000 ms during carotid sinus massage*
CM carotid sinus massage HBE=His bundle electrography CSRT=corrected sinus recovery time

Pat no	Longest R-R (ms)	Cardiac rhythm	Remarks
14	4 820	Complete AV block	Asymptomatic Li withdrawn repeat CM normal sinus rhythm
22	4 530	Asystole	Asymptomatic HBE + CSRT normal repeat CM R-R 1 560 ms
35	4 380	Asystole	Asymptomatic repeat CM R-R 2 360 ms Li induced sinus node dysfunction
47	8 760	Asystole	Asymptomatic repeat CM R-R 2 400 ms Li induced sinus node dysfunction
49	6 050	Complete AV block	Asymptomatic repeat CM normal sinus rhythm R-R 1 012 ms
101	4 340	Complete AV block	Asymptomatic digoxin + verapamil probably contributed

not exhibit any signs of sinus node disease when not on lithium It should however be borne in mind that the invasive electrophysiological methods available for sinus node investigation do not always disclose an existing dysfunction

The most probable explanation for the cardiac influence of lithium is that lithium replaces intra cellular potassium Lithium readily enters the cell during the depolarization phase but is not transported out again as effectively as sodium ions The inward potassium flux during the repolarization phase is therefore decreased, resulting in a supranormal extracellular potassium content and less potassium than normal inside the cell (7) These electrolyte changes may well explain the ST segment and T wave changes caused by lithium as well as the occurrence of premature beats Animal experiments have shown that lithium decreases the spontaneous rate of depolarization reduces the rate of propagation of electrical impulses and decreases the conduction velocity in the atrioventricular and intraventricular conduction system (7) Another contributory reason for the effects of lithium may be a reduction of the response to adrenergic stimulation of the heart

Since lithium is a relatively common drug in psychiatric treatment (6) it is important to be aware that it may induce dizziness and/or syncope caused by cardiac arrest Such symptoms may be overlooked and judged to be a manifestation of the psychiatric disorder The investigation performed to see whether sinus node dysfunction during lithium treatment is a common phenomenon did not suggest that it is Lithium treatment can obviously be instituted in all patients without a history suggesting sinus node dysfunction If there are any symptoms of dizziness and/or syncope lithium therapy should be postponed until a cardiological examination has been carried out Likewise a thorough cardiological examination should be performed if a patient on lithium develops symptoms of this type

REFERENCES

1 Code J F J Lithium salts in the treatment of psychotic exitement Med J Aust 36 349 1949
2 Demers R G & Heninger G R Electrocardiographic T wave changes during lithium carbonate treatment JAMA 218 381 1971
3 Eliasen P & Andersen M Sinoatrial block during lithium treatment Eur J Cardiol 3 97 1975
4 Kleinert M Myocardiopathie unter Lithiumterapie Med Klin 69 494 1974
5 Leading article Adverse effects of lithium treatment Br Med J 1 346 1977
6 Schon M Lithium in psychiatric therapy and prophylaxis J Psychiatr Res 6 67 1968
7 Singer J & Rotenberg D Mechanisms of lithium action N Engl J Med 289 254 1973
8 Swedberg K & Winblad B Heart failure as complication to lithium treatment Acta Med Scand 196 279 1974
9 Tangedahl T N & Gan G T Myocardial irritability associated with lithium carbonate therapy N Engl J Med 287 867 1972
10 Tilkian A G Schroeder J S Kao J & Hultgren H Effect of lithium on cardiovascular performance report on extended ambulatory monitoring and exercise testing before and during lithium therapy Am J Cardiol 38 701 1976
11 Tseng H L Interstitial myocarditis probably related to lithium carbonate intoxication Arch Pathol 92 414 1971
12 Wellens H J Manger Cats V & Duren D R Symptomatic sinus node abnormalities following lithium carbonate therapy Am J Med 59 285 1975
13 Wilson J R Krans E S Bailas M M & Rahita L Reversible sinus node abnormalities due to lithium carbonate therapy N Engl J Med 294 1223 1976 1976

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Hyperthermia and Rhabdomyolysis in Self-Poisoning with Paracetamol and Salicylates

Report of a Case

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ABSTRACT A young woman ingested large amounts of different analgesics, mainly salicylate and paracetamol On admission about 17 hours later, clearly toxic serum levels of both drugs were demonstrated She was comatose with respiratory failure for 5 days During the first day there was a period of several hours of therapy resistant hyperthermia A severe bleeding tendency was probably related to profound coagulation defects Persistingly elevated serum levels of ASAT and ALAT for two weeks were presumably caused by a toxic effect of paracetamol on the liver When consciousness was regained widespread pareses of skeletal muscles, predominantly of the lower limbs, were demonstrated These were related to extensive rhabdomyolysis as evidenced by extremely elevated serum levels of CPK for 6 weeks, and by muscle necrosis in biopsy specimens There was a gradual improvement, but walking disturbances were still present after one year The hyperthermia was probably related to the cerebral effects of salicylates or the combination of multiple drugs The rhabdomyolysis might be related to a deleterious effect of hyperthermia on the muscles or to an effect of paracetamol on the skeletal muscles similar to that which might occur in the myocardium, or to a combination of these mechanisms

Key words salicylates poisoning acetaminophen (paracetamol) poisoning fever liver disease chemically induced muscular diseases chemically induced (rhabdomyolysis)

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An overdose of paracetamol may in addition to liver damage cause myocardial necrosis (13 22 25 29) whereas rhabdomyolysis of skeletal muscles has not been reported Necrosis of skeletal muscles is known to occur in malignant hyperthermia and during heat stroke especially if associated with physical exertion (1 9) An overdose of salicylates may also induce hyperthermia (2 5 6 14) In these cases however rhabdomyolysis has not been reported This report concerns a case of self poisoning mainly with paracetamol and salicylates in which a period of hyperthermia was noted followed by extensive and persistent pareses owing to rhabdomyolysis

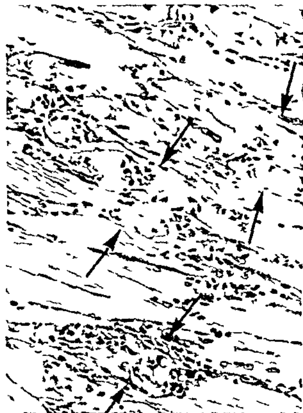
CASE REPORT

The patient a 21 year-old woman who had exhibited mild nervous symptoms previously had in recent years used bronchiolytic drugs for a moderate asthma She was admitted to our department on March 1 1976 16–18 hours after intake of a great number of different analgesics Retrospectively it was assumed that the ingested substances may have amounted to 70 g salicylates 25 g paracetamol 12 g phenazone 7 g caffeine 1 5 g codeine 1 g chlordiazepoxide and 0 5 g promethazine

On admission the patient was semicomatose excitable and hyperventilated Her temperature was 39 3 C BP 130/80 mmHg and she had a sinus tachycardia of 150/min Abundant liquid content with some tablet remnants was removed by gastric lavage and charcoal was deposited Laboratory tests showed a high plasma level of salicylate 856 μg/ml (28) and a combined respiratory alkalosis and metabolic acidosis The condition was interpreted as a heavy salicylate poisoning for which osmotic diuresis with urea and alkaline electrolyte solutions was started Initially a good diuresis was obtained and during the next 30 hours the plasma level of salicylate was reduced to 254 μg/ml Nevertheless she became deeply comatose during the first few hours

Seven hours after admission her condition deteriorated further with sudden development of hypotension oliguria and increase in rectal temperature which was continuously recorded with an electric thermometer Despite vigorous cooling with ice bags her temperature remained above 41 C for 5 hours and above 42°C for more than 3 hours the peak value of 43 3 C occurring 10 hours after

Abbreviations DIC = disseminated intravascular coagulation CPK = creatine phosphokinase



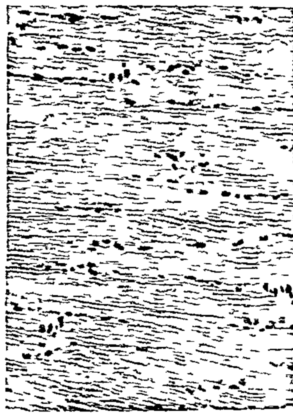


Fig 1 Biopsy from the right peroneus muscle on the 12th day after admission Left between arrows necrotic muscle fibres partly surrounded by leucocytes Right normal muscle fibres from the same tissue specimen

admission She was given plasma infusions and after 3 hours the clinical condition improved with the reestablishment of an adequate BP and diuresis and the body temperature normalized gradually

During the second day her condition deteriorated again owing to massive bleedings from the urinary and gastrointestinal tracts There were also bleedings from the nose and throat and widespread suggillations of the skin These bleedings were considered to be related to profound coagulation defects She was given vitamin K fresh whole blood transfusions cryoprecipitated plasma platelet concentrates and steroids Laboratory tests suggested disseminated intravascular coagulation (DIC) syndrome but heparin was not given Owing to atelectasis of the left lung mainly caused by aspirated blood a respiratory failure developed necessitating artificial ventilation for 8 days During the first half of this period she was given curare and kept unconscious with diazepine and morphine A concomitant pneumonia which was considered mainly responsible for a raised temperature of 38–39°C from the third to the 15th day in hospital was treated with penicillin cephalothin and tetracycline

On the fourth day pronounced elevations of ASAT 2 682 U/l (45 μkat/l) and ALAT 1 031 U/l (17 μkat/l) were noted whereas serum levels of bilirubin and alkaline phosphatases were normal Serial ECGs showed minor ST depressions most markedly on the third day The suspicion of liver damage caused by paracetamol was now aroused and this was confirmed by the finding of a toxic level of paracetamol 100 μg/ml (23) in a serum sample from the time of admission During the next 3 weeks there was a gradual and parallel reduction of the serum levels of ASAT and ALAT towards normal

As a prognostic index of a probable ischemic brain damage the content of creatine phosphokinase (CPK) in the cerebrospinal fluid was determined on the third and fourth day of admission Definitely elevated values of 141 and 131 U/l (2 4 and 2 2 μkat/l) respectively were noted Usually values above 10 U/l (0 17 μkat/l) in cerebrospinal fluid indicate severe brain damage Technique for determining isoenzymes of CPK was not available Extracerebral origin of the enzyme was considered and proved by the finding of an excessive elevated serum level of 27 615 U/l (460 μkat/l) on the sixth day of admission the upper normal level being 120 U/l (2 μkat/l) Serial determination of serum CPK revealed values above 2 000 U/l (33 μkat/l) for the next 2 weeks followed by a gradual decline to normal values after 6 weeks During this period she lost 13 kg in weight

Enzyme activities were estimated according to the Scandinavian standard methods

After 2 weeks the clinical condition slowly improved Now it became evident that there were widespread peripheral pareses of the skeletal muscles most prom

ment in the lower limbs Muscle biopsy from the right peroneus muscle performed on the 12th day revealed muscle necrosis (Fig 1) Electromyography performed on the 15th day showed a pattern of denervation consistent with some degree of neuropathy

The patient was discharged after 10 weeks and thereafter she stayed in a rehabilitation hospital for 8 months After one year she still had walking disturbances owing to bilateral pareses mainly of the foot extensors and of the hip joint adductors

DISCUSSION

Of the seven substances ingested the two most prominent were salicylate and paracetamol which were both present in the serum at toxic levels on admission most likely higher levels had been present before admission Salicylates may induce dysfunction of the platelets In addition both drugs may have contributed to the pronounced bleeding tendency by interfering with the production of coagulation factors in the liver (2 4 5 14) DIC was probably present too a process which is known to occur in hyperthermic states (10 21) In combination with chlordiazepoxide both drugs may account for the cerebral dysfunction associated with an element of transitory ischemic brain injury The liver was not enlarged but the markedly elevated serum levels of ASAT and ALAT are in accordance with liver damage caused by paracetamol (3 8) Myocardial necrosis is known to occur during paracetamol poisoning (13 22 25 29) and the enzyme levels with consistently higher values for ASAT than ALAT may indicate that the enzymes partly originated from the myocardium ECG however gave no indication of myocardial necrosis The extremely and persistingly elevated enzyme levels are most consistent with liver damage which was also substantiated by the low levels of the coagulation factors

The main clinical and biochemical features of this patient were the few hours of therapy resistant hyperthermia occurring shortly after admission the excessively and persistently raised serum levels of CPK the pronounced signs of muscular necrosis proved by biopsy and the long lasting pareses of the muscles of the lower limbs The pathogenesis of the hyperthermia and the rhabdomyolysis is not obvious Muscle necrosis may be caused by hyperthermia (1 9 11) Raised temperature may be a feature of salicylate poisoning but muscular necrosis has not been reported in this condition Neither hyperthermia nor necrosis of peripheral muscles have been reported in paracetamol poisoning The latter may however be suspected since myocardial necrosis is known to occur in paracetamol poisoning The probability therefore exists that hyperthermia related to a toxic effect from salicylate may have caused the muscular damage in this patient or may have rendered peripheral muscles susceptible to a toxic effect from paracetamol

REFERENCES

1 Brinkmann B & Puschel K Zur Histomorphologie der Herz und Skelettmuskulatur bei maligner Hyperthermie Z Rechtsmed 80 117 1977
2 Done A K & Temple A R Treatment of salicylate poisoning Mod Treatm 8 528 1971
3 Editorial Paracetamol hepatotoxicity Lancet 2 1189 1975
4 Gazzard B G Henderson J M & Williams R Early changes in coagulation following a paracetamol overdose and a controlled trial of fresh frozen plasma therapy Gut 16 617 1975
5 Goodman L S & Gilman A The pharmacological basis of therapeutics 5th ed pp 325–339 Macmillan New York 1975
6 Havill J H Malignant hyperthermia caused by salicylate overdose associated with phenelzine therapy — a case report Anaesth Intensive Care 2 380 1974
7 Hill J B Salicylate intoxication N Engl J Med 288 1110 1973
8 James O Lesna M Roberths S H Pulman L Douglas A P Smith P A & Watson A J Liver damage after paracetamol overdose Lancet 2 579 1975
9 Knochel J P Environmental heat illness Arch Intern Med 133 841 1974
10 — Disseminated intravascular coagulation in heat stroke JAMA 231 496 1975
11 Knochel J P & Schlein E M On the mechanism of rhabdomyolysis in potassium depletion J Clin Invest 51 1750 1972
12 Koch Weser J Acetaminophen N Engl J Med 295 1297 1976
13 Maclean D Peters T J Brown R A G McCathie M Baines G F & Robertson P G C Treatment of acute paracetamol poisoning Lancet 2 849 1968
14 McCleave D J & Havill J A review of acute salicylate poisoning Anaesth Intensive Care 2 340 1974
15 Metha A C & Baker R N Persistent neurological deficits in heat stroke Neurology 20 336 1970
16 Mitchell J R Jollow D J Potter W Z Gillette J R & Brodie B B Acetaminophen induced hepatic necrosis IV Protective role of glutathione J Pharmacol 187 211 1973
17 Mitchell J R Thorgeirsson S S Potter W Z

Jollow D J & Keiser H Acetaminophen induced hepatic injury Protective role of glutathione in man and rationale for therapy Clin Pharmacol Ther 16 676 1974

18 Moulds R F W & Denborough M A Biochemical basis of malignant hyperpyrexia Br Med J 2 241 1974

19 Newson A J Malignant hyperthermia Three case reports N Z Med J 75 138 1972

20 O Donnell T F & Clowes G H A The circulatory abnormalities of heat stroke N Engl J Med 287 734 1972

21 Perchick J S Winkelstein A & Shadduck R K Disseminated intravascular coagulation in heat stroke Response to heparin therapy JAMA 231 480 1975

22 Pimstone B L & Uys C J Liver necrosis and myocardiopathy following paracetamol overdosage S Afr Med J 42 259 1968

23 Prescott L F Gas liquid chromatographic estimation of paracetamol J Pharm Pharmacol 23 807 1971

24 Rush J L & Foltz E L Malignant hyperthermia J Neurosurg 46 385 1977

25 Sanerkin N G Acute myocardial necrosis in paracetamol poisoning Br Med J 3 478 1971

26 Sonnenklar N & Krasna I H Clinical management of malignant hyperpyrexia J Pediatr Surg 11 617 1976

27 Stanley B & Pal N R Fatal hyperpyrexia with phenelzine and imipramine Br Med J 2 1011 1964

28 Trinder P Rapid determination of salicylate in biological fluids Biochem J 57 301 1954

29 Weston M J & Williams R Paracetamol and the heart Lancet 1 536 1976

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Blood Pressure Reduction and Vascular Adaptation

A Study on Long Term Effects of Treatment with Mefruside or Atenolol

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ABSTRACT Systemic BP reduction, calf blood flow and vascular resistance in the calf were determined in forty two previously untreated patients with mild to moderate essential hypertension (WHO I–WHO II) before and after 6 weeks, 6 months and 18 months of BP lowering treatment with mefruside (25 mg daily) or atenolol (100–400 mg daily) Blood flow was determined with venous occlusion plethysmography using a mercury in rubber strain gauge technique in the supine patient Auscultatory BP was measured on the right arm simultaneously with the flow determinations and resistance was calculated from the flow and pressure BP was reduced significantly and to the same extent by the two drugs In the atenolol group a rise in resting resistance and a corresponding fall in resting blood flow was seen initially These changes were entirely normalized during continued treatment for 18 months In the mefruside group no significant haemodynamic changes during treatment were observed at rest apart from the BP fall None of the drugs reduced resistance at 'maximal vasodilatation, indicating that no regress of the hypertensive structural changes of the calf blood vessels had taken place

Key words hypertension arteriolar hypertrophy reversibility atenolol mefruside
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Haemodynamic studies indicate that blood flow resistance in established hypertension is increased mainly because of structural changes in the resistance vessels Thus an increased blood flow resistance was observed in peripheral vascular beds also during maximal vasodilatation in patients with established essential hypertension (4 5 15) and in spontaneously hypertensive and renal hypertensive rats (6 7 12) Furthermore the dose–response curve to noradrenaline given i a was steeper than normal both in man and in animals (6 7 12, 15) Maximal blood pressure (BP) response under constant flow conditions was also increased in vascular beds of hypertensive rats indicating an enhanced contraction force in the vascular wall (6 7 12) The most likely explanation for these haemodynamic findings in hypertension is an increased wall thickness in relation to the lumen in the resistance vessels (15) Morphological support for this view has been given (8 18) The vascular wall of small arteries and arterioles seems to be thickened partially because of smooth muscle hypertrophy and partially because of increased amounts of other components

Animal experiments show that the blood vessels as judged from the haemodynamic parameters can be completely normalized by early BP lowering treatment (12 20) On the other hand if therapy is started in the middle aged or old animal reversibility is incomplete (20) Observations in man are so far somewhat contradictory Thus studies on hand blood flow resistance indicated some reversibility of the changes after 5 years of BP lowering therapy (11 16) whereas no change was observed in the calf blood vessels after 6 months of treatment (16) Further in patients with aortic coarctation haemodynamic studies indicated that morphological vascular changes in the forearm still remained 10 years after successful surgical correction of the anomaly with elimination of the BP gradient at rest (14)

The aim of the present investigation was to further elucidate the question of reversibility of vascular changes in essential human hypertension and to study whether treatment with diuretics or β adrenoceptor blocking agents would influence the vascular changes differently as judged by studies of peripheral haemodynamics

Table I *Patient data (mean ± S E M)*

	Atenolol group	Mefruside group
Men/women	16/3	20/3
Age (y)		
Mean	46±2 8	52±2 0
Range	23–58	29–63
Height (cm)	176±1 4	176±1 6
Weight (kg)		
Men	82±3 2	85±2 3
Women	60±6 4	65±2 9
WHO I/WHO II	9/10	8/15

Differences between the groups were insignificant

SUBJECTS AND METHODS

Forty two patients 6 women and 36 men 23–63 years of age were included in the study The patients all of whom were referred to the Out Patient Hypertension Clinic at the Sahlgren s Hospital had mild to moderate BP elevation (WHO stage I–II) All had essential hypertension as judged from our routine clinical investigation (2)

The patients were allocated at random to one of two groups so that 23 were treated with mefruside 25 mg daily and 19 with atenolol 100–400 mg (mean 272 mg) daily There were no significant differences between the two groups concerning BP or other clinical findings before treatment (Tables I and II)

A control group comprised 14 healthy normotensive subjects 11 men and 3 women They were significantly younger than the patients (mean age 35 7±8 8 years range 27–51)

Calf blood flow and forearm BP were determined in the patients before and after 6 weeks 6 months and 18 months of BP lowering therapy and in the untreated controls only once Blood flow in both calves was measured plethysmographically with the subject in the supine position A modified Whitney mercury in rubber strain gauge technique was used (21) One BP cuff was applied just proximal to the knee and one around the ankle Cuff pressure during flow registration was about 55 mmHg in both cuffs Forearm BP was determined with the ordinary cuff method For diastolic BP Korotkoff sound phase 5 (disappearance) was used Mean pressure was estimated as diastolic BP plus one third of the pulse pressure Resistance was calculated from simultaneously measured mean blood flow Blood flow and resistance were first determined after 30 min of supine rest These parameters were then reexamined during intense maximal vasodilatation induced by a combination of arterial ischaemia (proximal cuff inflated to well above the systolic BP and calf muscle work until exhaustion) The flow and resistance values in the tables and figures are calculated from individual mean values based on six determinations three in each leg In order to exlude arteriosclerotic occlusions of the big arteries of the legs oscillometry (Gesenius-Keller) was performed in all patients at the thigh calf and ankle levels

Standard methods were used for calculation of the mean the standard error and the linear correlation coefficient The hypothesis of no difference in means within groups was tested with Student s *t* test while the *t* test for independent data was used between groups The hypothesis of no difference in proportion was tested with Wilcoxon s test and χ^2 test Two-tailed tests were used and $p<0\ 05$ was considered significant

RESULTS

In addition to increased BP both patient groups showed significantly elevated blood flow resistance during maximal vasodilatation compared to the controls (Fig 1) Maximal blood flow as well as the oscillometry recordings were normal in both patient groups

The mean BP reduction after 18 months of treatment was significant in both treatment groups (Ta

Table II *Resting BP in the recumbent position at the Hypertension Clinic before and during treatment with atenolol or mefruside (mean ± S E M)*

	Atenolol group (*n*=19)	Mefruside group (*n*=23)	Significance of difference between groups	Change in BP during treatment		Significance of difference between groups
				Atenolol group	Mefruside group	
Before treatment						
Systolic	179±3 1	172±3 1	n s			
Diastolic	104±2 7	103±1 3	n s			
After 6 months treatment						
Systolic	145±3 9	147±2 8	n s	34±4 5	25±3 6	n s
Diastolic	88±2 1	93±1 7	n s	16±3 6	10±2 0	n s
After 18 months treatment						
Systolic	138±4 1	142±3 1	n s	41±4 9	30±3 8	0 1>*p*>0 05
Diastolic	84±2 9	90±1 9	*p*<0 05	20±4 4	13±2 0	n s

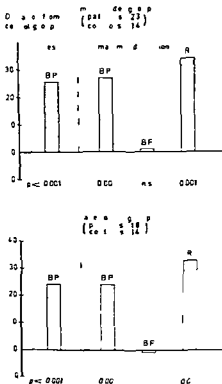


Fig. 1. Comparison of indirectly measured mean arterial pressure (*BP*), calf blood flow (*BF*) and vascular resistance of the calf (*R*) during maximal dilatation between healthy volunteers and hypertensives before treatment with mefruside or atenolol.

ble II) 41/20 mmHg in patients on atenolol and 30/13 mmHg in those on mefruside. The difference in BP reduction was not significant.

Changes in blood flow and blood flow resistance during treatment both at rest and during vasodilatation are shown in Fig. 2. In the mefruside group no significant changes were found during resting conditions. During maximal dilatation a significant drop in blood flow corresponding to the BP reduction was observed after six weeks and it persisted throughout the observation period. The resistance at maximal vasodilatation did not change during the observation period.

A significant reduction of blood flow at rest was observed in the atenolol treated group already after six weeks and it persisted during the examination after six months (Fig. 2). After 18 months the resting blood flow had returned to pretreatment levels. Resting vascular resistance showed an increasing trend reaching significance after six months. The vascular resistance at rest was normalized after 18 months. During vasodilatation a corresponding trend towards an increased resistance was seen after six months but this increase was not statistically significant. Thus neither of the drugs changed resistance during maximal vasodilatation significantly and no reduced resistance during dilatation was seen in either group. Even when those

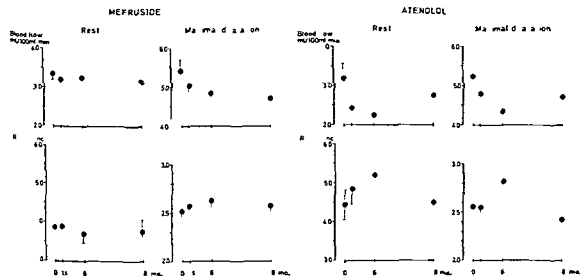


Fig. 2. Changes in resting blood flow (*BF*) and resistance (*R*) at rest and during maximal dilatation in patients treated with atenolol or mefruside for 6 weeks, 6 months and 18 months.

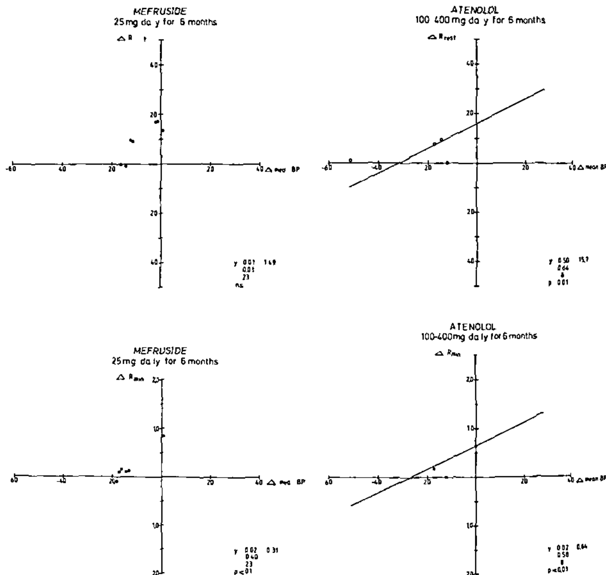


Fig 3 Change in blood flow resistance at rest (R_{rest}) and during maximal vasodilatation (R_{min}) plotted against change in mean auscultatory BP during 6 months of treatment with mefruside or atenolol

patients who showed the most pronounced BP fall during therapy (Δ mean arterial BP ≥10 mmHg) were analyzed separately no significant change in resistance during vasodilatation was seen in either group

The resting blood flow was significantly more reduced in the atenolol than in the mefruside group both at six weeks and at six months but not after 18 months The change in resting resistance differed significantly between the two groups after six months but not after six weeks or 18 months

A significant correlation between the changes in BP and resistance at six months both at rest and during vasodilatation was found in the atenolol but not in the mefruside group (Fig 3) No such correlations existed after 18 months

DISCUSSION

The vasodilatation induced by ischaemia combined with calf muscle work until exhaustion is very intense Before treatment the dilatation procedure increased the blood flow from 3 3 to 53 and 54 ml/min 100 ml respectively in the two patient groups (Fig 2) We have observed that during stepwise increments in work duration the blood flow in

creases until 3–4 min duration but then levels off indicating a state close to maximal vasodilatation after 4–5 min However a slight increase in flow might be seen at the highest work load levels and complete smooth muscle relaxation in the resistance vessels is thus probably not always obtained Therefore it should be kept in mind that a submaximal work load due to e g poor patient cooperation could result in a submaximal vasodilatation and consequently in submaximal blood flow as measured by the present method Experiments on forearm blood vessels show that ischaemic work in that vascular bed can fully eliminate the effect of noradrenaline given i v (5) thus indicating that the present method induces a very intense vasodilatation Furthermore isoprenalinesulphate when given i a into the femoral artery in large doses (1 6–2 9 μg/min) could not dilate the calf blood vessels to the same extent as the method used in the present study Nor could a combination of drug infusion and ischaemic work dilate the calf blood vessels more than ischaemic work alone (17) However substantial doses of noradrenalinebitartrate (1 6–2 9 μg/min) given i a into the femoral artery could not be fully overridden by the procedure for dilatation of the calf vessels used in the present study (17) The present data thus indicate that ischaemic work induces an almost complete relaxation of the resistance vessels of the calf But it is also evident that intense vasoconstrictor stimuli can still to a small extent influence resistance during dilatation Furthermore resistance during maximal dilatation might be influenced by changes in work intensity

In agreement with previous observations on other vascular beds in animal and man (4 5 6 7 12 15) calf vascular resistance was increased during vasodilatation in hypertensive patients compared to normotensive controls (Fig 1) This finding supports the opinion that established human hypertension is distinguished by generalized haemodynamically important structural changes in the small resistance vessels Normal oscillometry in the legs of all patients indicated that the increased resistance in the patient groups was not due to arteriosclerotic occlusions of the big leg arteries

The BP reduction during treatment was convincingly demonstrated in both groups (Table II) Some influence on BP reduction due to adjustment to the clinic cannot be ruled out but the reduction was of the same magnitude as could be expected from a one-drug regimen (1 3 10)

The most striking haemodynamic change induced by the treatment apart from the BP reduction was the temporary rise in resting resistance in the atenolol group and the corresponding fall in blood flow (Fig 2) This observation is in agreement with previously observed changes in the central haemodynamic picture induced by non selective β adrenoceptor blocking drugs (9 19) but in our series the reduction of resistance with time occurred later

Long term studies have shown that in contrast to non selective β blocking agents selective β_1 adrenoceptor blockers such as atenolol change total peripheral resistance very little if at all (1 13) The reason for the different findings in the present study and the haemodynamic studies mentioned above may possibly be different dosages the drug having a significant effect also on β_2-adrenoceptors in higher dosages Alternatively there may be regional differences between vascular beds

The positive correlation between the changes in resting resistance and BP corresponds very well to the observations by Tarazi and Dustan on the central haemodynamic changes during propranolol treatment (19) The existence of a positive correlation indicates that the main cause of the rise in resistance during treatment with β adrenoceptor blocking agents cannot be a simple baroreceptor response If so one would rather expect a negative correlation the rise in resistance being more pronounced the more the pressure falls However a baroreceptor response which initially affects every patient and then decreases with time in some individuals but not in others cannot be excluded

During vasodilatation reduction of BP and blood flow was seen in both groups after 6 and 18 months treatment the latter being mainly secondary to the drop in BP (Fig 2) No significant change in resistance was observed in any group Resistance during vasodilatation tended in fact to increase after six months in the atenolol group The explanation for this trend and for the positive correlation between changes in BP and resistance during vasodilatation (Fig 3) is not clear It is not likely that the trend indicates a structural change in the vessels In fact the same trend was observed already 24 hours after administration of propranolol or atenolol as indicated by a few preliminary model experiments in healthy individuals A more likely explanation is that the dilatation is submaximal and that factors inducing the rise in resistance at rest can also affect

resistance during dilatation An alternative explanation could be that the β adrenoceptor blocker because of its metabolic effects and its effect on tenacity for physical work reduces the maximal metabolite concentration and therefore the degree of vasodilatation In view of the normalized resistance during vasodilatation occurring after 18 months a submaximal dilatation is the most tempting explanation

No reduction of resistance during vasodilatation was seen in the course of long term treatment in any group and thus no change was observed indicating reversibility of structural vascular changes The reason why this study on calf blood vessels unlike a previous investigation on hand blood vessels (16) showed no signs indicating reversibility of structural vascular changes might be that different vascular beds were studied Because of man s upright position the calf blood vessels will be exposed to a higher BP than the vessels in the hands Therefore the structural vascular changes may be more severe with e g more extensive collagen invasion and therefore less reversible (20) Regional differences may thus exist Further the duration of treatment was shorter and the degree of BP reduction smaller in the present than in the previous study (16)

Based on our present and previous experiences we therefore feel that hypertensive structural vascular changes in established human hypertension are partially but not completely reversible with BP lowering therapy

REFERENCES

1 Amery A Billiet L Joossens J V Meekers J Reybrouck T & van Mieghem W Preliminary report on the haemodynamic response of hypertensive patients treated with beta blocker (ICI 66082) Acta Clin Belg 28 358 1975

2 Andersson O Berglund G Hansson L Sannerstedt R Sivertsson R Wikstrand J & Wilhelmsen L Organization and efficacy of an outpatient hypertension clinic Acta Med Scand 203 391 1978

3 Bergstrom J Hultman E & Solheim S B The effect of mefruside on plasma and muscle electrolytes and blood pressure in normal subjects and in patients with essential hypertension Acta Med Scand 194 427 1973

4 Conway J A vascular abnormality in hypertension A study of blood flow in the forearm Circulation 27 520 1963

5 Folkow B Grimby G & Thulesius O Adaptive structural changes of the vascular walls in hypertension and their relation to the control of the peripheral resistance Acta Physiol Scand 44 255 1958

6 Folkow B Hallback M Lundgren Y & Weiss L Structurally based increase of flow resistance in spontaneously hypertensive rats Acta Physiol Scand 79 373 1970

7 — Background of increased flow resistance and vascular reactivity in spontaneously hypertensive rats Acta Physiol Scand 80 93 1970

8 Furuyama, M Histometrical investigations of arteries in reference to arterial hypertension Tohoku J Exp Med 76 388 1962

9 Hansson L Beta adrenergic blockade in essential hypertension Acta Med Scand (Suppl) 550 1973

10 Hansson L Åberg H Karlberg B E & Westerlund A Controlled study of atenolol in treatment of hypertension Br Med J 2 367 1975

11 Hansson L & Sivertsson R Reversibility of structural vascular changes in human essential hypertension Pathophysiology and management of arterial hypertension Proceedings of a conference held in Copenhagen Denmark April 10–11 1975 Lindgren & Soner Molndal 1975

12 Lundgren Y Adaptive changes of cardiovascular design in spontaneous and renal hypertension Haemodynamic studies in rats Acta Physiol Scand (Suppl) 408 1974

13 Lund Johansen P Haemodynamic long term effects of a new β adrenoceptor blocking drug atenolol (ICI 66082) in essential hypertension Br J Clin Pharmacol 3 445 1976

14 Samánek M Geotzová J Fiserova J & Skovranek J Differences in muscle blood flow in upper and lower extremities of patients after correction of coarctation of the aorta Circ Res 54 347 1976

15 Sivertsson R The hemodynamic importance of structural vascular changes in essential hypertension Acta Physiol Scand (Suppl) 343 1970

16 Sivertsson R & Hansson L Effects of blood pressure reduction on the structural vascular abnormality in skin and muscle vascular beds in human essential hypertension Clin Sci Mol Med 51 77 1976

17 — Unpublished observation 1977

18 Suma N & Takahashi T Morphological and morphometrical analysis of circulation in hypertension and ischaemic kidney Urban & Schwarzenberg Munchen Berlin and Wien 1971

19 Tarazi R C & Dustan H P Beta adrenergic blockade in hypertension Practical and theoretical implications of long term hemodynamic variations Am J Cardiol 29 633 1972

20 Weiss L Aspect of the relation between functional and structural cardiovascular factors in primary hypertension Experimental studies in spontaneously hypertensive rats Acta Physiol Scand (Suppl) 409 1974

21 Witney R J The measurement of volume changes in human limbs J Physiol 121 1 1953

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Mortalıty and Morbıdıty durıng 13 5 Years' Follow-up ın Relatıon to Blood Pressure

The Study of Men Born ın 1913

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ABSTRACT A total of 973 50 year-old men, ran domly selected from the general population ın Goteborg, Sweden, were invited to a survey ın 1963 Altogether 855 men (88%) participated They have been followed up for 13 5 years and this report pre sents mortality and morbidity during this time ın relation to BP at the time of the initial examination BP was strongly associated with mortality regardless of its cause This was due to a close relation of BP to mortality from ischaemic heart disease and a weak but significant relation to mortality from cancer BP was also strongly related to morbidity from myocar dial infarction, stroke and angina pectoris, showed a tendency towards relation to morbidity from inter mittent claudication and kidney stone, and was nega tively related to morbidity from neoplasm for sur vivors BP was also related to pension but not to sick leave There is no indication of a decreasing impor tance of BP as a risk factor ın this age period

Key words blood pressure myocardial infarction stroke

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It has long been known that hypertensives have an excess morbidity and mortality from certain dis eases It ıs only during recent years however that the magnitude of the problem has been fully under stood (19 24 34 39) This report presents mortality and morbidity data from the study of men born ın 1913 which was started ın 1963 The cohort has been followed up for 13 5 years

STUDY POPULATION AND METHODS

All inhabitants of Sweden have a national registration number that includes their date of birth and other vital statistics Names addresses and registration numbers are registered by the County Census Bureau and were acces sible before the sample was drawn for the present study The study population was recruited from men living ın Goteborg Sweden who were born ın 1913 and were still alive at the age of 50 years (1963) All men meeting these criteria who were born on a date divisible by three—the 3rd 6th 9th day (and so on) of each month—comprised the study sample 973 men met these criteria Of these 855 (88%) agreed to be examined ın 1963 at Sahlgrenska Sjukhuset Goteborg (45) Of the 118 non participants 7 had died 4 were hospitalized and 9 had moved from the city at the time of the investigation 40 men consented to examination ın their homes The remaining 58 men re fused to participate for various reasons usually because of a negative attitude towards medical care Compared to participants non participants had a lower mean income were more frequently unmarried and were more often registered by the Temperance Board Slightly more non participants than participants had received sickness ben efit (46) During 13 5 years of follow up the 40 non par ticipants examined at home had the same mortality pattern as the participants while the 78 men refusing examination had a higher mortality rate (43)

Of the 855 men examined ın 1963 792 were re-examined ın 1967 and 703 of these men were re examined once again ın 1973 (Fig 1) Of the 855 men 15 did not participate ın 1967 but were re examined ın 1973 Of those who partici pated ın 1963 and ın certain cases ın 1967 26 were not able to attend the examination ın 1973 but replied to a postal questionnaire of the same type as that answered by the men who attended the examination

BP was measured ın 1963 as the casual BP ın the right arm ın the seated position after 5 min rest A mercury manometer with a cuff size of 12×23 cm was used The same observer performed all measurements The pressure was read to the nearest 5 mmHg as systolic BP when the Korotkoff sounds were first heard and as diastolic BP phase 4 at muffling and phase 5 when the sounds disap-

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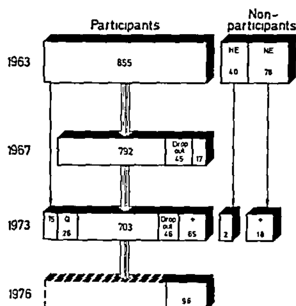


Fig 1 Composition of the population in the study of men born in 1913 *HE* = home examination group *NE* = not examined + = deaths

peared completely Only the systolic and diastolic phase 4 pressure will be presented in this report The systolic–diastolic BP classification for the 855 men in 1963 shown in Fig 2 will be used in this presentation The sample has been subdivided into 4 groups according to systolic and diastolic BP so as to yield an extreme low pressure group of 5% two intermediate groups of 70 and 20% and an extreme high pressure group consisting of 5% of the sample (the heavy lines in Fig 2) The division along the two axes forms a chequered matrix in which the squares are mbered according to the figure The squares have then been grouped as follows 1 2–5 6 7–15 16 The population ellipse in the figure has thus been divided into 5 groups along its longitudinal axis

All men who had a systolic BP of ≥ 175 mmHg and a diastolic BP of ≥ 115 mmHg at one of the examinations and at a subsequent control measurement were offered antihypertensive treatment Table I shows the number of men treated in relation to the total number of men investigated in the various BP groups at the three occasions In 1963 14 of the 855 men were on treatment at the time of the investigation against 77 out of 744 men examined in 1973 In 1963 6 out of 39 men (15%) in group 16 were receiving treatment against 27 out of 29 (93%) in 1973

Mortality data for all the 973 men in the original sample were followed up continuously during the study by scrutiny of death certificates and periodic checks on vital statistics with parish offices (the authority which has primary responsibility for the census register) or the corresponding authority for men resident abroad The follow up was 100% In this report mortality data up to 1976 have been used corresponding to 13 5 years of follow up on average Death certificates were available for all men who died and 83% had been autopsied

Morbidity data for stroke and myocardial infarction up to 1976 for all 855 men followed up were obtained by interview death certificates and from Nov 1968 on wards from the Myocardial Infarction (9) and Stroke Register (12) These registers cover the city of Goteborg and include more than 94% of all infarcts and strokes Special arrangements were made for men who had moved out of the area The criteria of stroke were hospitalization with a diagnosis of stroke or fresh cerebral thrombosis or haemorrhage at the postmortem examination Subarachnoidal or subdural haemorrhage was not included The criteria of myocardial infarction were hospitalization with a clinical diagnosis of infarction or postmortem findings of fresh coronary heart disease The clinical criteria of myocardial infarction were those adopted by the Swedish Society of Cardiology central chest pain shock or syncope suggesting myocardial infarction together with a typical transaminase spectrum and/or appearance of a pathological Q wave or localized ST variations in the ECG The criteria for coronary heart disease at the postmortem examination were a fresh myocardial scar or total or almost total occlusion of a coronary artery and a medical history suggesting myocardial infarction

Morbidity data for intermittent claudication kidney stone gallbladder disease pancreatitis neoplasm diabetes gastric and duodenal ulcers and gastrointestinal haemorrhage were collected by interview Morbidity data for angina pectoris were obtained by means of a questionnaire Men who reported having had chest pain or retrosternal oppression at any time were subjected to a case history evaluation for classification of the pain as typical or suspected angina pectoris or other chest pain according to Rose s criteria (35)

Data concerning pensions were obtained by means of a questionnaire in 1973 Information on sick leave during 1955–73 for all 973 men in the orginal sample was obtained

Table I *Treatment of hypertension at each examination by BP group in 1963 (no of men treated in relation to no of men examined)*

Year of examination	BP groups in 1963 1	2–5	6	7–15	16	Total
1963	0/26	0/63	1/469	7/258	6/39	14/855
1967	0/26	0/56	2/434	18/239	25/35	45/792
1973	0/21	0/59	12/410	38/225	27/29	77/744

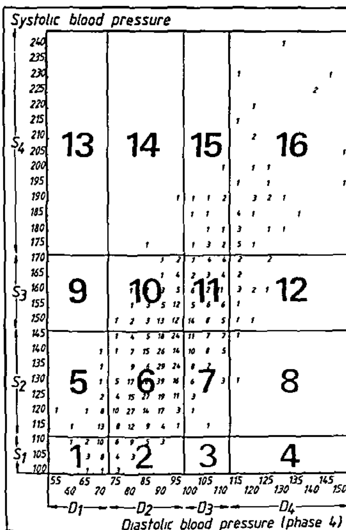


Fig 2 Systolic and diastolic BPs for the 855 men examined in 1963 The men have been divided into 4 groups according to both systolic and diastolic BP The resulting 16 groups have then been numbered as shown in the figure Non existing squares (3 and 4) and empty squares (9 and 13) are indicated for the sake of clarity

from the National Health Insurance Office which covers everybody resident in Sweden

Statistical methods

For analysis of differences in mortality morbidity retirement and length of sick leave between different BP groups a test of the linear trend in $2\times n$ tables was used The test is equivalent to Fisher s permutation test where the groups are classified according to a nominal scale as 0 1 2 etc (28) The test takes into account differences between groups only when the differences represent a trend in one or the other direction A standard technique was used for the life table constructions

RESULTS

Mortality

Of the 855 men who participated in the investigation in 1963 96 died during the 13 5 year follow up period Table II shows the number of deaths by diagnosis and diagnostic groups and the incidence in the form of the annual death rate per 10000 individuals for each diagnosis and diagnostic group by systolic BP in 1963 The incidence rate for ischaemic heart disease increased from 0 deaths per 10000 men per annum in the lowest pressure group to 93 in the highest The trend was consistent and significant ($p=0\ 006$) The diagnostic group was subdivided into myocardial infarction and other ischaemic heart diseases For myocardial infarction the same strong correlation to systolic BP was found whereas no clear trend could be demonstrated for the subgroup other ischaemic heart diseases Stroke exhibited a convincing trend but owing to the small number of deaths the correlation

Table II. *Incidence rate (cases/10000 men/y) for mortality in the systolic BP groups and 13.5 years follow up of 855 men aged 50 at entry*

Cause of death	n	Systolic BP in 1963 (mmHg) <115	115–145	150–170	≥175	All	p≤
Ischaemic heart disease	37	0	30	31	93	32	0.006
Myocardial infarction	30	0	25	18	93	26	0.009
Other	7	0	5	13	0	6	ns
Stroke	4	0	1	4	26	3	ns
Cancer	26	12	18	40	26	23	ns
Lung	10	12	5	18	13	9	ns
Other	16	0	13	22	13	14	ns
Other causes	29	12	25	31	26	25	ns
Accidents, violence	6	0	8	0	0	5	ns
Suicide, intoxication	8	12	5	4	26	7	ns
Miscellaneous	15	0	12	22	13	13	ns
All causes	96	25	74	111	159	83	0.001
Persons at risk		60	572	167	56	855	

was not significant. Cancer also tended to be correlated to systolic BP but the relationship was not consistent in the highest BP group and was not statistically significant. The incidence rate for other causes of death also tended to increase with increasing systolic BP, but the relationship was not statistically significant. When all causes of death were grouped together, the incidence rate increased significantly with increasing BP.

The result was the same for diastolic BP except that the trends for stroke and cancer were significant ($p \leq 0.05$) and there was no longer a rising trend for other causes. In Table III the men have been grouped by both systolic and diastolic BP in accordance with Fig. 2. Table III gives the incidence rate in consecutive BP groups. Ischaemic heart disease, myocardial infarction and cancer were significantly correlated to systolic–diastolic BP. A strong trend was found for stroke.

The three methods of grouping the men by BP thus gave essentially similar results. The systolic–diastolic BP classification gave a good balance be

Table III. *Incidence rate (cases/10000 men/y) for mortality in the systolic–diastolic BP groups and 13.5 years follow up of 855 men aged 50 at entry*

Cause of death	n	BP groups in 1963: 1	2–5	6	7–15	16	All	p≤
Ischaemic heart disease	37	0	0	28	37	114	32	0.001
Myocardial infarction	30	0	0	24	26	114	26	0.001
Other	7	0	0	5	11	0	6	ns
Stroke	4	0	0	2	3	38	3	ns
Cancer	26	0	12	17	37	19	23	0.05
Lung	10	0	12	6	11	19	9	ns
Other	16	0	0	11	26	0	14	ns
Other causes	29	28	35	21	29	38	25	ns
Accidents, violence	6	0	24	5	3	0	5	ns
Suicide, intoxication	8	28	0	6	6	19	7	ns
Miscellaneous	15	0	12	9	20	19	13	ns
All causes	96	28	47	68	106	209	83	0.001
Persons at risk		26	63	469	258	39	855	

Table IV *Incidence rate (cases/10 000 men/y) for morbidity from first myocardial infarction and morbidity from first stroke in the systolic–diastolic BP groups and 13.5 years follow up of 855 men aged 50 at entry*

		BP groups in 1963						
End point	*n*	1	2–5	6	7–15	16	All	$p \leq$
First myocardial infarction	73	0	47	50	78	220	64	0 001
Survival	50	0	47	31	60	120	44	0 001
Death	23	0	0	19	17	100	20	0 005
Case fatality rate (%)	23/73	–	0	38	22	45	31	
Stroke	21	0	12	6	23	156	18	0 001
Myocardial infarction and/or stroke	88	0	47	56	92	340	77	0 001
Myocardial infarction and/or stroke during 13 5 years follow up (%)		0	6 3	7 6	12 4	45 9	10 4	

tween the two factors and this method of classification only is therefore used below

Morbidity

Table IV shows that the incidence rate for morbidity from myocardial infarction i e the occurrence of primary myocardial infarction was strongly correlated to BP in 1963 for both fatal and non fatal infarction The case fatality rate i e the proportion of infarction patients who die from their first infarction tended to increase with increasing pressure The incidence rate for stroke (primary events) was also strongly correlated to BP in 1963 Table IV also gives the morbidity from myocardial infarction and/or stroke as the percentage morbidity during the 13 5 year period No patient in the extreme low pressure group had an event compared to 46% of the patients in the extreme high pressure group

Incidence figures for angina pectoris intermittent claudication kidney stone gallbladder disease pancreatitis neoplasm diabetes gastric and duodenal ulcer and gastrointestinal haemorrhage could be calculated for the 744 individuals who were re examined in 1973 or who answered the questionnaire Table V shows the incidence rate during the 10-year period 1963–73 for these diseases classified by systolic–diastolic BP in 1963 As the number of individuals in group 1 was so small this group has been pooled with groups 2–5 The incidence rate for angina pectoris intermittent claudication kidney stone and to some extent diabetes tends to increase with the systolic–diastolic BP in

Table V *Incidence rate (cases/10 000 men/y) for specified diseases in the systolic–diastolic BP groups and 10 years follow up of 744 men aged 50 at entry and still alive at the end of the period*

A negative *p* value indicates a trend towards an inverse relationship

		BP groups in 1963					
	n	1–5	6	7–15	16	All	$p \leq$
Angina pectoris	74	90	106	92	185	103	ns
Intermittent claudication	26	38	27	36	138	35	ns
Kidney stone	35	40	48	54	120	52	0 06
Gallbladder disease	38	55	63	37	71	55	ns
Pancreatitis	5	0	12	0	0	7	ns
Neoplasm	15	76	15	14	0	20	–0 01
Diabetes	21	13	20	54	0	29	ns
Peptic ulcer	18	18	34	25	0	28	ns
Gastrointestinal haemorrhage	7	14	13	5	0	10	ns
All	186	450	311	309	381	327	ns
Persons at risk		79	412	224	29	744	

Table VI *Prevalence (cases/1000 men) for angina pectoris in the systolic–diastolic BP groups sub-grouped according to presence of angina in 1963 1967 and 1973 and 10 years follow up of 703 men aged 50 at entry and still alive at the end of the period*

Angina pectoris	n	BP groups in 1963 1–5	6	7–15	16	All	p≤
In 1963 1967 and 1973	8	0	14	5	83	12	ns
In 1967 and 1973	14	0	14	29	120	21	0 005
In 1973 only	56	91	28	61	83	80	ns
Intermittent	21	28	38	25	0	32	ns
All	99	114	141	112	241	133	ns

1963 None of the relationships is statistically significant however The incidence rates for neoplasm and gastrointestinal haemorrhage both tended to decrease with decreasing BP the trend for neoplasm being statistically significant ($p<0\ 01$)

Table VI shows the results of a more detailed analysis of the relationship between angina pectoris and BP The 703 men for whom follow up data were available from 1963 1967 and 1973 were classified according to the reported prevalence of angina pectoris at the time of the three investigations Table VI thus gives the prevalence for angina pectoris in 1963 1967 and 1973 angina in 1967 and 1973 only angina in 1973 only and angina intermittently i e in 1963 only and not in 1967 or 1973 in 1963 and 1973 only and not in 1967 etc The prevalences of *in 1963 1967* and *1973* and in *1967* and *1973* tended to increase with increasing BP in 1963 the increase in the group with angina in 1967 and 1973 being statistically significant ($p<0\ 005$) No trend was found for the prevalence of angina in 1973 only or intermittent angina.

Retirement and sick leave

The incidence rates for disability pensions and other pensions during 1963–73 are shown in Table VII The incidence rate for both types of pension tends to increase with increasing BP in 1963 Together the two trends are statistically significant ($p<0\ 05$) Table VIII shows the average number of days of illness and periods of sick leave in relation to BP in 1963 In the upper half of the table only sick leave not leading to a disability pension is included No definite trend was found Regression analysis using individual values revealed a weak insignificant negative trend for both methods of measuring morbidity In the lower half of the table sick leave resulting in a disability pension during the 10-year period is included There is still no definite trend

Life table analysis

All these analyses have also been performed using the life table method Fig 3 shows the results presented as cumulative frequencies for death irrespective of cause infarction morbidity stroke morbidity and retirement The results are essentially the same as in Tables III IV and VII The main point of interest in Fig 3 is the time course for the different events For death and myocardial infarction there is a fairly linear differentiation of the five BP groups For stroke there is little difference between the groups during the first two years after

Table VII *Incidence rate (events/10000 men/) for disability and other pensions in the systolic–diastolic BP groups and 10 years follow up of 703 men aged 50 at entry*

	n	BP groups in 1963 1	2–5	6	7–15	16	All	p≤
Disability pension	54	56	61	64	98	148	77	0 08
Other pensions	42	0	40	61	70	36	60	ns
All kinds of retirement	96	56	102	126	168	185	138	0 05

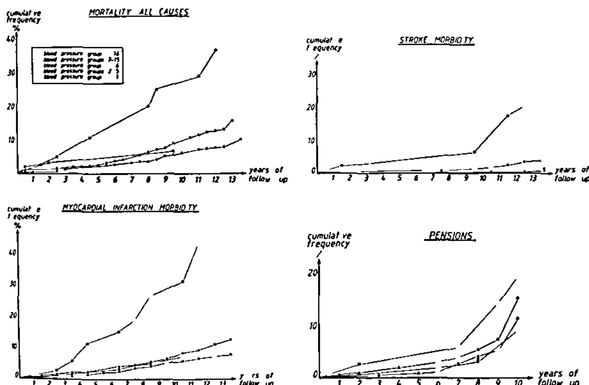


Fig 3 Cumulative frequencies in relation to time of death irrespective of cause infarction morbidity stroke morbidity and retirement in the Study of Men Born in 1913 The analysis was performed using the life table method

which the highest group deviates For retirement the groups show the same pattern during the first six years and then diverge somewhat

DISCUSSION

The investigation took the form of a cohort study in which a random sample of 50-year old men from the general population was subjected to 13 5 years observation for mortality and morbidity from myocardial infarction and 10 years follow up in respect of other data The drop out was of two types—primary in 1963 and gradual during the course of the study Of the 118 men constituting the primary drop-out those 40 who were examined in their homes had the same mortality and morbidity pattern as the 855 participants (43) and there is

Table VIII *Average number of days of sick leave and periods of sick leave in the systolic–diastolic BP groups*

	BP groups in 1963						
	1	2–5	6	7–15	16	All	$p\leq$
Mean no /10 years for surviving non retired men							
Days of sick leave	167	270	179	157	193	179	n s
Periods of sick leave	9 4	10 8	8 2	8 6	11 0	8 6	n s
Mean no /10 man years for surviving men calculated by life table method							
Days of sick leave	179	318	226	253	268	241	n s
Periods of sick leave	9 4	11 8	8 8	9 5	10 1	9 3	n s

therefore no reason to suppose that this drop-out influenced the results of the study The remaining 78 men in the primary drop-out group had a higher cardiovascular and non cardiovascular mortality (43) and thus probably had other cardiovascular characteristics than the participants The group is so small however that it does not influence the results even if its characteristics are essentially different from those of the participants

The secondary drop out which only influences the analysis of 10-year data consisted of 45 individuals in 1967 and 46 in 1973 The distribution by systolic BP in 1963 has previously been presented for both these groups (43) It does not differ markedly from that of the participants in either drop-out group The drop out groups are also small The 744 men followed up in 1973 constitute 94% of the 790 survivors from the 1963 investigation and 84% of the 889 survivors from the original sample This drop-out is therefore unlikely to have had any marked influence on the results

A large number of studies have shown that elevated BP is associated with excess mortality (3–6 15 16 19 20 24 30 40) Many studies, like the present one have also shown that the relationship is progressive the risk for death increasing with increasing BP A threshold level at which the BP ceases to be normal and becomes pathological does not exist the risk increases at a somewhat increasing rate from the lowest pressure upwards The excess mortality is largely due to excess mortality cardiovascular disease above all myocardial ~~rction sudden unexpected death and stroke (3 15 16 19 24 30 40) Several studies have demonstrated a relationship between BP on the one hand and the degree of atherosclerosis at postmortem examination (1 10 27) heart failure (1 19) and aortic aneurysm (19 34) on the other Excess mortality from cancer (8 17 33) nephritis influenza pneumonia and diseases of the digestive system (24) in hypertensive individuals has also been reported

The correlation between BP and excess morbidity from coronary heart disease (3 6 7 16 19 20 24 26 29 30 31 37 41 47) and stroke (6 14 16 20 22, 24 30 38 41) has also been well documented The importance of elevated BP as a risk factor for myocardial infarction and stroke does not seem to decline with age up to 70–77 years (19 20 38) In the Framingham study the case fatality rate for coronary heart disease increased with increasing BP (19) as it also tended to do in the present study Several studies have also demonstrated a correlation between BP and reinfarction among patients with primary infarction (11, 32 51) and between BP and congestive heart failure (21) and claudication (19)

Most of the studies quoted have included myocardial infarction and angina pectoris in the concept coronary heart disease Investigations in which the components have been studied separately have shown the same correlation to BP for both of them (13 19 36) When the men with angina pectoris in this study were divided up into those with periods without chest pain and those who had continuous chest pain a significant correlation to BP was found in the latter but not in the former This means that the anginal diagnosis probably is contaminated with other types of chest pain not only in this study but in all probability also in other studies and the correlation between angina pectoris and BP is probably stronger than the data in the literature would suggest

High BP is thus associated with a massive excess mortality and morbidity It is therefore not surprising that hypertensives more often receive disability pensions than other people (6 23) The weak trend for other pensions is probably due to the fact that certain occupational groups are able to retire without their retirement being classified as being due to disability It may seem more surprising however, that hypertensives do not have more sick leave than other people, as was also found in a cross sectional study of SAAB Scania employees (25)

How did antihypertensive treatment influence the results of this study? There is reason to believe that treatment influenced the mortality and morbidity from cerebrovascular disease congestive heart failure and renal damage (44 48 49 50) The treatment might also have had a beneficial effect on morbidity and mortality from coronary heart disease (2 42) even if no randomized trial has been able to indicate such an effect As shown in Table I the scale of antihypertensive treatment in the study population in 1963 was modest and reflected the scale of antihypertensive treatment in the general population at that time Since then it has undoubtedly been improved but it may be assumed that the group studied was subjected to more intensive antihypertensive treatment than groups with corresponding BPs in the general population Since there is reason to assume that the treatment had a certain preventive effect on morbidity and mortality related

to hypertension the excess morbidity and mortality demonstrated in this study probably represents an underestimation of that in the general population

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REFERENCES

1 Baker A B Resch J A & Loewenson R B Hypertension and cerebral atherosclerosis Circulation 39 701 1969
2 Berglund G Wilhelmsen L Sannerstedt R Hansson L Andersson O Sivertsson R & Wikstrand J Decrease of CHD morbidity by treatment of hypertension Lancet 1 1 1978
3 Borhani N O Hechter H H & Breslow L Report of a ten year follow up study of the San Francisco longshoremen Mortality from coronary heart disease and from all causes J Chron Dis 16 1251 1963
4 Build and blood pressure study 1959 vol. I and II Society of Actuaries 1959
5 Deubner D C Tyroler H A Cassel J C Hames C G & Becker C Attributable risk population attributable risk and population attributable fraction of death associated with hypertension in a biracial population Circulation 52 901 1975
6 Dimond G E Hypertension body weight and coronary heart disease Arch Intern Med 112 550 1963
7 Dunn J P Ipsen J Elsom K O & Ohtani M Risk factors in coronary artery disease hypertension and diabetes Am J Med Sci 259 309 1970
8 Dyer A R Stamler J Berkson D M Lindberg H A & Stevens E High blood pressure a risk factor for cancer mortality? Lancet 1 1051 1975
9 Elmfeldt D Wilhelmsen L Tibblin G Vedin J A Wilhelmsson C E & Bengtsson C Registration of myocardial infarction in the city of Goteborg Sweden J Chron Dis 28 173 1975
10 Evans P H Relation of longstanding blood pressure levels to atherosclerosis Lancet 1 516 1965
11 Frank C W Weinblatt E Shapiro S & Sager R V Prognosis of men with coronary heart disease as related to blood pressure Circulation 38 432 1968
12 Harmsen P & Tibblin G A stroke register in Goteborg Sweden Acta Med Scand 191 463 1972
13 Heyden S Bartel A G Tabesh E Cassel J C Tyroler H A Cornoni J C & Hames C G Angina pectoris and the Rose questionnaire Arch Intern Med 128 961 1971
14 Heyman A Karp H R Heyden S Bartel A Cassel J C Tyroler H A & Hames C G Cerebrovascular disease in the biracial population of Evans County Georgia Arch Intern Med 128 949 1971
15 Holme I & Waaler H T Five year mortality in the city of Bergen Norway according to age sex and blood pressure Acta Med Scand 200 229 1976
16 Inter society commission for heart disease resources—Artherosclerosis study group and epidemiology study group Primary prevention of the atherosclerotic diseases (ed I S Wright and D T Fredrickson) Cardiovascular diseases—Guidelines for prevention and care US Government Printing Office Washington D C 1974
17 Jenkin R D T & Stryker J A The influence of the blood pressure on survival in cancer of the cervix Br J Radiol 41 913 1968
18 Kagan A Gordon T Kannel W B & Dawber T R. Blood pressure and its relation to coronary heart disease in the Framingham study Hypertension 7 53 1958
19 Kannel W B Role of blood pressure in cardiovascular morbidity and mortality Prog Cardiovasc Dis 17 5 1974
20 — Role of blood pressure in cardiovascular disease The Framingham study Angiology 26. 1 1975
21 Kannel W B Castelli W P MacNamara, P M McKnee P A & Feinleib M Role of blood pressure in the development of congestive heart failure The Framingham study N Engl J Med 287 781 1972
22 Kannel W B Wolf P A Verter J & McNamara, P M Epidemiologic assessment of the role of blood pressure in stroke The Framingham study JAMA 214 301 1970
23 Lerner P R Social security disability applicant statistics 1969 US Department of Health Education and Wellfare Social Security administration Office of Research and Statistics DHEW Publication no (SSA) 74-1911 US Government Printing Office Washington D C 1973
24 Lew E A High blood pressure other risk factors and longevity the insurance viewpoint Am J Med 55 281 1973
25 Malmgren S & Andersson G På jakt efter halsoprofilen. C1 och D1 uppsats i sociologi Linkopings universitet 1976
26 Mathewson F A L Brereton C C Keltie W A & Paul G I The University of Manitoba Follow up Study a prospective investigation of cardiovascular disease Part II Build blood pressure and electrocardiographic factors possibly associated with the development of coronary heart disease Can Med Ass J 92 1002 1965
27 Murata, K. Terasawa, F Hosoda S Ikeda M & Seki, M Relation of blood pressure and serum total cholesterol to severity of atherosclerotic lesions in aorta, coronary and cerebral arteries Jpn Heart J 12 460 1971
28 Oden, A & Wedel H Arguments for Fisher s permutation test. Ann Stat 3 518 1975
29 Paffenbarger R S Notkin J Krueger D E Wolf P A Thorne M C LeBauer E J & Williams J L Chronic disease in former college students II Methods of study and observations on mortality from coronary heart disease Am J Public Health 56 962 1966

30 Paul O Risks of mild hypertension a ten year report Br Heart J (Suppl) 116 1971
31 Pell S & D Alonzo C A A three year study of myocardial infarction in a large employed population JAMA 175 463 1961
32 — Immediate mortality and five year survival of employed men with a first myocardial infarction N Engl J Med 270 915 1964
33 Reserpin and breast cancer Report from the Boston Collaborative Drug Surveillance Program Boston University Medical Center Lancet 2 669 1974
34 Roberts W C The hypertensive diseases Evidence that systemic hypertension is a greater risk factor to the development of other cardiovascular diseases than previously suspected Am J Med 59 523 1975
35 Rose G A & Blackburn H Cardiovascular survey methods pp 172-175 WHO 1968
36 Rosenman R H Friedman M Straus R Jenkins C D Zyzanski S J & Wurm M Coronary heart disease in the Western Collaborative Group Study J Chron Dis 23 173 1970
37 Rosenman R H Friedman M Straus R Wurm M Jenkins C D & Messinger H B Coronary heart disease in the Western Collaboratory Group Study A follow up experience of two years JAMA 195 130 1966
38 Shekelle R B Ostfeld A M & Klawans H L Jr Hypertension and risk of stroke in an elderly population Stroke 5 71 1974
39 Stamler J Hypertension and coronary risk implications of current knowledge Acta Cardiol (Brux) (Suppl) 20 1974
40 Stamler J Berkson D M & Lindberg H A Risk factors their role in the epidemiology and pathogenesis of the atherosclerotic diseases In Pathogenesis of atherosclerosis (ed R W Wissler and J C Geer) p 41 Williams & Wilkins Baltimore 1972
41 Stamler J Lindberg H A Berkson D M Shaffer A Miller W & Poindexter A Epidemiological analysis of hypertension and hypertensive disease in the labor force of a Chicago utility company Hypertension 7 23 1958
42 Svardsudd K Berglund G & Tibblin G Morbidity and mortality in treated and untreated hypertension results from the Goteborg 50-year-old men study Drugs (Suppl) 1 34 1976
43 Svardsudd K & Tibblin G A longitudinal blood pressure study Blood pressure change during ten years in relation to initial value The study of men born in 1913 J Chron Dis To be published
44 Taguchi J & Freis E D Partial reduction of blood pressure and prevention of complications in hypertension N Engl J Med 291 329 1974
45 Tibblin G High blood pressure in men aged 50—a population study of men born in 1913 Acta Med Scand (Suppl) 470 10 1967
46 — A population study of 50-year-old men An analysis of the non participation group Acta Med Scand 178 453 1965
47 Tyroler H A Heyden S Bartel A Cassel J C Cornoni J C Hames C G & Kleinbaum D Blood pressure and cholesterol as coronary heart disease risk factors Arch Intern Med 128 907 1971
48 Veterans Administration Cooperative Study Group on Antihypertensive Agents Effects of treatment on morbidity in hypertension Results in patients with diastolic blood pressures averaging 115 through 129 mmHg JAMA 202 1028 1967
49 — Effects of treatment on morbidity in hypertension II Results in patients with diastolic blood pressure averaging 90 through 114 mmHg JAMA 213 1143 1970
50 — Effects of treatment on morbidity in hypertension III Influence of age diastolic pressure and prior cardiovascular disease further analysis of side effects Circulation 45 991 1972
51 Wilhelmsson C , Vedin J A Elmfeldt D Tibblin G & Wilhelmsen L Hypertension and myocardial infarction J Chron Dis 31 157 1978

Acta Med Scand 205 493–500 1979

Aggregation of Deaths from Ischaemic Heart Disease among First and Second Degree Relatives of 108 Males and 42 Females with Myocardial Infarction

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ABSTRACT The occurrence of deaths due to ischaemic heart disease (IHD) among first and second degree relatives of coronary patients and among relatives of an equal number of matched controls was studied on the basis of death certificates The propositi were 108 males and 42 females, who had developed myocardial infarction (MI), males before 65 years of age and females before 70 When compared with controls, a 1 1/2-fold higher death rate was found among first degree relatives of the propositi and a lower average age at death The difference in death rate between second degree relatives was not significant, except for maternal brothers When compared with the death rate due to IHD in the general population, the increase in risk to first degree relatives of propositi was nearly 3-fold over the expected value, and 1 1/2-fold to second degree relatives The age of the propositi at onset of MI did not affect the risk to relatives, whereas sex related influence was significant To first degree relatives of male propositi the risk increase was up to 5 fold over the expected value The risk increase was found to be greatest to first degree male relatives of female propositi or over 7 fold to fathers and brothers Mothers and sisters of both male and female propositi showed a 4–5-fold risk increase over the expected value The risk increase to second degree relatives was 2 1/2-fold over the expected value to maternal brothers of male propositi and 4-fold to such brothers of female propositi The relatives of controls showed a coronary mortality close to that of the general population Familial clustering of coronary deaths was found in 8 7% of the families of the propositi and in 4 7% of the families of the controls The findings of the present study indicate a substantial genetic component in the overall aetiology of IHD, which is more prominent in families of female propositi but hardly of a magnitude to warrant genetic counseling

Key words aggregation IHD death risk relatives familial clustering

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The relatively few systematic studies on familial aggregation of ischaemic heart disease (IHD) have mostly been restricted to first degree relatives of male coronary patients Results of these studies have shown a statistically significant aggregation of IHD deaths among first degree relatives of index patients when compared either with matched controls or the general population The excess mortality from IHD among parents of index males (14) and fathers and siblings of index males under the age of 65 years (10), and an increased risk of IHD deaths in younger first degree relatives of coronary patients i e males under 55 years and females under 65 years of age (17) suggest a genetic component in the aetiology of the disease Twin studies (1 3 6 7 8) have shown a higher concordance rate of IHD among monozygotic twins of the same sex than among dizygotic pairs which indicates a substantial genetic influence

This susceptibility is strongly sex linked and influenced by other well known and in part genetically determined risk factors e g hypertension and familial hypercholesteraemia as well as by less well known environmental factors common to near relatives Familial aggregation of IHD among relatives of index males has been reported by several authors in spite of insufficient documentary evidence of morbidity and mortality from IHD among relatives (2 5 9 16 19) More extensive data are needed on aggregation of IHD among relatives of index female coronary patients and among relatives of patients more distant than first degree

The objective of the present communication is to report on death rate and increased risk of death from IHD among first and second degree relatives

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Abbreviations IHD = ischaemic heart disease MI = myocardial infarction

Table I *Classification and number of relatives of propositi and controls before and after exclusion of deaths before 1951*

Classification of relatives	Relatives of propositi			Relatives of controls			Total		
	Before exclusion	After exclusion	Difference	Before exclusion	After exclusion	Difference	Before exclusion	After exclusion	Difference
1st degree	886	745	141	779	667	112	1 665	1 412	253
2nd degree	1 044	716	328	1 126	779	347	2 170	1 495	675
Propositi/controls									
Male	1 402	1 092	310	1 434	1 136	298	2 836	2 228	608
Female	528	369	159	471	310	161	999	679	320
Male relatives	966	706	260	926	668	258	1 892	1 374	518
Female relatives	964	755	209	979	778	201	1 943	1 533	410
Total	1 930	1 461	469	1 905	1 446	459	3 835	2 907	928

of 150 coronary patients compared with age- and sex matched relatives of equal numbers of controls and with the general population

SUBJECTS AND METHODS

Propositi The 150 patients 108 males and 42 females of Icelandic descent came with a few exceptions from Reykjavik and its neighborhood They had been hospitalized due to acute myocardial infarction (MI) proven by clinical evidence ECG and in most cases by raised serum transamninases In 133 cases the first attack of MI had been diagnosed at Reykjavik City Hospital and in 17 cases at other hospitals the majority of cases being referred to hospitals in 1965–75 The males selected were all under 65 years of age and the females under 70 at the onset of their first MI There was no consanguinity among the patients

Controls A control matched by age and sex was selected for each of the 150 propositi 96 were drawn from the medical ward of the City Hospital 54 were healthy subjects The selection criteria were no awareness of IHD and no consanguinity between themselves or the propositi Seventy of the male controls and 61 of the male propositi were manual workers No further attempts were made to match propositi and controls

Table II *Age of propositi at onset of MI (category 420) kinship and number and percentage of deaths due to IHD (categories 420–422) among relatives (% of certain cases)*

P = paternal M = maternal

	Relatives of propositi				Relatives of controls			
	Certain cases (*N*)	Not IHD (*N*)	IHD (*N*)	Deaths (%)	Certain cases (*N*)	Not IHD (*N*)	IHD (*N*)	Deaths (%)
Age of propositi at onset of MI (y)								
<45	347	286	61	17 58*	348	306	42	12 07
45–54	352	295	57	16 19	378	328	50	13 23
55–64/69	303	246	57	18 81*	313	275	38	12 14
Kinship								
Fathers	86	59	27	31 40 } *	98	80	18	18 37
Brothers	91	69	22	24 18 } *	73	60	13	17 81
Mothers	109	91	18	16 51 } *	105	94	11	10 48
Sisters	55	45	10	18 18 } *	51	44	7	13 73
P brothers	171	142	29	16 96	166	146	20	12 05
M brothers	176	134	42	23 86	161	138	23	14 29
P sisters	148	133	15	10 14	205	187	18	8 78
M sisters	166	154	12	7 23	180	160	20	11 11
Total	1 002	827	175	17 47 *	1 039	909	130	12 51

* $p<0.05$ ** $p<0.01$ * All components added but not the subgroups show $p<0.05$ significance

Table III *Risk (per year) of dying from IHD in Iceland by calendar and age periods*

Age (y)	1951–55	1956–60	1961–65	1966–70	1971–75	1951–75
20–29	0	0	0	0	0	0
30–39	0 0001	0	0 0001	0 0001	0 0001	0 0001
40–49	0 0003	0 0006	0 0006	0 0006	0 0006	0 0006
50–59	0 0010	0 0016	0 0020	0 0023	0 0026	0 0020
60–69	0 0033	0 0038	0 0049	0 0062	0 0057	0 0049

Relatives The selected IHD patients and controls were linked to their relatives by means of computerized genealogical data compiled by the Genetic Committee of the University of Iceland These records include the date and cause of death All deaths were documented by death certificates Excluded from the study were grandparents and relatives deceased before 1951 (the first year of the study period) as well as relatives under 20 years of age and half siblings After exclusions the total number of relatives in the study was 2907 (Table I)

General populatoin During the study period (1951–70) the population increased from 145 604 to 204 578 with a birth rate declining from 27 5 to 21 5‰ and a crude death rate around 7‰ (11 12) In this period IHD has been the major cause of death the mortality rate from the disease being the lowest in Northern and Western Europe (15) Until recent times the socio-economic level has been low but uniform On the whole the present population has a common background being either children or grand children of manual workers The population is rather homogeneous as regards race and status and thus provides an encouraging field for hereditary studies

Death certificates In 1951 the Sixth Revision of the International Classification of Diseases Injuries and Causes of Death was adapted in Iceland followed by later revisions Since then practically all death certificates have been issued and signed by medical doctors and checked for correctness of the code number by the same person (J Sigurjonsson) before being entered in the Statistical Bureau s Death Register During 1951–60 and 1961–75 the autopsy rate in Iceland was 23% and 35 7% respectively (12 18) Almost 2/3 of all deaths in the present study occurred in hospitals or other institutions 38% of the causes of death having been confirmed by post mortem examinations all performed at the Department of Pathology University of Iceland

Definitions IHD Classified according to the 1955 revision of WHO International Statistics Classification of Diseases Injuries and Causes of Death *Not IHD* Columns thus headed give number of individuals dead after 1950 of other causes than IHD and individuals alive over 64/69 years of age *Certain cases* Number of individuals after exclusion of uncertain cases i e individuals dead before 1951 and individuals alive under 64/69 years of age

Statistical analysis The individual records of families of propositi and controls were analysed and computed at the Computer Center University of Iceland Computer programs were designed in Fortran IV on IBM S 360/30 and on PDP 11/34 Statistical methods and tests used are standard and commonly known

RESULTS

Table I shows the general character of the sample as a whole Exclusion of deaths before 1951 affects the two groups rather similarly

Death rates from IHD among relatives of propositi and controls

In the youngest age group shown in Table II there are 43 male and 7 female propositi/controls in the next 37 males and 13 females and in the oldest group 28 males and 22 females Table II shows higher death rates among relatives of the propositi in the sample as a whole and also among first degree rela

Table IV *Observed (O) and expected (E) number of IHD deaths among relatives of propositi by age and age periods and O/E ratio*

Age (y)	1951–55			1956–60			1961–65			1966–70			1971–75			1951–75		
	O	E	O/E	O	E	O/E	O	E	O/E	O	E	O/E	O	E	O/E	O	E	O/E
20–29	0			0			0			0			0			0		
30–39	0			0			0			0			0			0		
40–49	0			2			2			1			0			5		
50–59	3	1 59	1 89	6	2 37	2 53*	4	2 53	1 58	6	2 30	2 61	8	2 31	3 46 *	27	12 16	2 22 **
60–64/69	10	3 42	2 92*	8	4 00	2 00*	12	5 11	2 35 *	13	6 34	2 05	11	4 89	2 25*	54	24 66	2 19* *

p<0 1 * p<0 01 ** p<0 001

Table V *Effect of age of propositi at onset of MI sex of propositi and relatives kinship and sex propositi on risk to relatives of dying from IHD*

E = expected O = observed IHD deaths

	Age of relative (y)	No of man years at risk	No of IHD deaths E	No of IHD deaths O	O/E ratio
Age of propositi at onset (y)					
≤54	50–59	4 430	8.74	16	1.83*
	60–64/69	3 730	18.30	41	2.24 **
>54	50–59	1 719	3.40	11	3.24***
	60–64/69	1 281	6.28	13	2.07*
Relatives of male propositi					
Male	50–59	2 163	7.14	15	2.10**
	60–64	1 312	9.45	20	2.12**
Female	50–59	2 501	1.75	5	2.86*
	60–69	2 483	6.95	12	1.73*
Relatives of female propositi					
Male	50–59	727	2.40	4	1.67
	60–64	426	3.07	16	5.21***
Female	50–59	758	0.53	3	5.66
	60–69	800	2.24	6	2.68
Relatives of propositi					
Male	50–59	4 664	9.22	20	2.17 *
	60–64/69	3 795	18.60	32	1.72*
Female	50–59	1 485	2.93	7	2.39
	60–64/69	1 226	6.01	22	3.66***
Relatives of male propositi					
1st degree	50–59	2 435	4.81	15	3.12 *
	60–64/69	1 284	6.28	12	1.91*
2nd degree	50–59	2 229	4.39	5	1.14
	60–64/69	2 506	12.27	20	1.63
Relatives of female propositi					
1st degree	50–59	963	1.90	5	2.63
	60–64/69	603	2.95	15	5.08 **
2nd degree	50–59	522	1.03	2	1.94
	60–64/69	623	3.06	7	2.29

$^{*}p<0.1$ $^{**}p<0.01$ * $p<0.001$

tives but in the subgroups among maternal brothers only The figures in the other subgroups are too small to reach statistical significance but in the case of the basic figures for parents and brothers the difference is apparent though not proven ($p>0.05$ $p<0.10$) The average age at death was significantly lower among first degree and female relatives of the propositi than among the corresponding relatives of controls

Risks of death from IHD to relatives of propositi and controls

Table III shows the IHD risk in the Icelandic population defined as the number of IHD deaths divided by the number of man years in each calendar and age period Between 1951 and 1975 a total of 257 IHD deaths were recorded in various age group between 20 and 69 years (11 12 18) in a total o 2417775 man years As an example from Table III the age group 60–69 and the calendar period 1971 75 show a risk of 57/10000 per year This resul is calculated as 396 IHD deaths divided by 6962 man years Table III shows that calendar period can be grouped together but the age periods can not be treated as a whole The table is used to fin the expected numbers of IHD deaths among rela tives of propositi and for statistical tests in com parison with the general population

In Table IV the man years at risk and IHD death among relatives of propositi are classified by ag

Table VI *Effect of kinship sex of relatives and sex of propositi on risk to relatives of dying from IHD*

O=observed E=expected IHD deaths P=paternal M=maternal

	Age of relatives (y)	O/E ratio for relatives of propositi	
		Male	Female
1st degree relatives			
Male	50–59	3 09**	2 19
	60–64	1 66	7 49***
Female	50–59	4 26*	5 27*
	60–69	2 93*	4 98**
2nd degree relatives			
Male	50–59	1 15	1 01
	60–64	2 35**	3 76 *
Female	50–59	1 23	6 61
	60–69	1 12	
Detailed kinskip			
Fathers	50–59	1 59	6 99
	60–64	2 81*	7 72**
Mothers	50–59		
	60–69	4 45	5 07*
Brothers	50–59	3 41* *	1 63
	60–64	0 63	7 38***
Sisters	50–59	5 58**	5 99
	60–69	0 96	4 94**
P brothers	50–59	1 31	2 40
	60–64	1 83	3 27
P sisters	50–59	2 83	
	60–69	1 00	
M brothers	50–59	1 03	
	60–64	2 73**	3 99**
M sisters	50–59		10 65*
	60–69	1 09	

$p<0\ 1$ $^{**}p<0\ 01$ $^{***}p<0\ 001$

and calendar periods In all 86 IHD deaths were observed among 20476 man years The reduction from 175 in the original data to 86 is due to the age and calendar period restrictions necessary for comparison with the general population Table IV shows that calculations must be restricted to age groups 50–59 and 60–64/69 The expected number of IHD deaths are used together with the observed number to find the ratios of observed to expected numbers among relatives of propositi Significance results from the Poisson tests clearly show that first and second degree relatives of coronary patients have more than a 2 fold increase in risk of death from IHD compared with the general population

Table V explains itself Table VI shows the effect of kinship and sex distribution of propositi on risk of death in relatives The very high increase in maternal sisters of female propositi is not significant, as only one IHD death was observed in the age group 50–59 and 134 man years at risk Fathers of female propositi had 54 man years and 3 IHD deaths in the age group 60–64

The risk to relatives of controls showed a difference from the general population which approached marginal significance

Familial clustering of IHD deaths

Thirteen of the propositi (6 males and 7 females) and 7 of the controls (4 males and 3 females) had two or more relatives who had died from IHD before the age of 64/69 The relatives of the 6 male propositi were 10 males and 5 females and of the 7 female propositi 7 males and 8 females The corresponding numbers of relatives of male controls were 3 males and 2 females and of the female controls 3 males and 6 females In the propositi group 21 IHD deaths occurred among first degree and 9 among second degree relatives the respective numbers for the control group being 9 and 5

The number of relatives alive or dead from other causes than IHD was 41 for propositi and 20 for controls Of the 6 male propositi previously mentioned 4 were under 55 years and had no relatives who had died from IHD under that age Of the 7 female propositi 4 were under 65 with 3 male relatives dead before 55 and 5 females before 65 years Among the controls 1 male under 45 had a male relative who had died of IHD before 45 all other relatives of controls died after the age of 55 years Both parents of a female propositus aged 42 died from IHD at 61 and 63 years of age two brothers under 55 are well

DISCUSSION

The general validity of this study depends on the absence of serious bias between the two samples to be compared It is evident from the nature of the samples that a deliberate selection was necessary

Table II shows some variation between number of fathers brothers and paternal sisters The difference in number of fathers is due to deaths before 1951 Thirty of the propositi and 27 of the controls either had half brothers or none at all Nineteen brothers of the propositi and 24 brothers of the con

trols were excluded from the pedigree because of age Thirteen fathers of the propositi either had half sisters or none and 49 sisters had died before 1951 The corresponding numbers for control fathers were 20 and 53 None of the controls was aware of having a disease known to carry risk of IHD One the other hand three of the propositi (two males aged 46 and 54 and one female aged 37) had coincidental diabetes Seven of the propositi (2 males and 5 females all over 54 years old) had diastolic hypertension One among the relatives of the propositi and three among the relatives of the controls died suddenly They were included as no other reasonable explanation for their death than IHD was found at post mortem examination

No great differences in the socio economic status of the families of propositi and controls were apparent Both groups were predominantly of urban or semiurban residence Parents and grandparents on both sides were with a few exceptions either farmers fishermen or other manual workers As socio-economic matching between groups like these is bound to be approximate it was not attempted It is not unreasonable to assume that family members in both groups are representative of the same population and that comparison is therefore justified It should be added however that the groups selected were from the Reykjavik area and may therefore not be completely representative for the other parts of the country

The question of familial aggregation of IHD has been approached either by comparing morbidity or mortality of relatives of index patients with that of matched controls or with the aid of life tables to find the ratio between the observed numbers of death due to IHD among relatives of index patients and the expected numbers (age adjusted death rate) in the general population When evaluating the outcome of the studies reported methodologic differences must be considered Index patients are likely to be more aware than controls of a similar disease in relatives The age structure of the samples and the number of relatives incorporated varies from one study to another Documentation of morbidity or mortality among relatives is often scant or lacking This has been overcome in some studies by a sufficient number of death certificates (3 8 10 13 14 17) and also by clinical examination of the relatives (13) Post mortem confirmation of the causes of death is reported in two studies (3 8)

Our data are consistent with the findings of previous authors as regards familial aggregation of IHD deaths among first degree relatives of coronary patients but differ in some respects regarding the degree of death rates Table II shows a 1 1/2 fold increase in the mortality rate among first degree relatives of propositi of all ages (both sexes) compared with controls while other studies which are based on male index cases of different ages and reports of history of IHD in relatives of index cases and controls show a mortality rate 2–4 times higher among the first degree relatives of young and middle aged male index patients compared with relatives of controls (2 5 9 14) Slack and Ewans (17) have reported a 5 fold increase in risk of death from IHD over the expected value to first degree male relatives of male index patients under 55 years and a 2 1/2 fold increase in risk to first degree female relatives under 65 compared with the general population as well as a 6–7 fold increase in risk to the first degree relatives of both sexes of female index patients The risk to relatives of the older index patients was not significantly above the expected value The relatives of the controls showed a coronary mortality close to that of the general population Rissanen and Nikkila (13) have reported a more than 5 fold risk of dying from IHD before the age of 65 for fathers of index males under 56 years of age and 5 5 times greater risk for brothers of index males than for brothers of controls The corresponding risk for sisters of the index patients was 2 5 times greater than for sisters of controls The differences between the results of our life table analysis and the findings of previous authors (13 17) may be due to differences in age structure (Table VI) and in the relatively high average age at death from IHD among first degree relatives in our study (Table VI) Table VI shows a less marked increase in risk to first degree relatives of the male propositi under 55 years of age but a more marked to female relatives The increase in risk to both sexes of first degree relatives of the female propositi in our series is consistent with that reported by Slack and Ewans (17) Table V shows on the other hand that the risk to relatives of the older propositi is significantly above the expected value

A clustering of IHD in families (two or more cases probands excluded) has been reported by Rissanen and Nikkilä (13) who found clustering among one quarter of families of 104 males with angina pectoris Slack and Ewans (17) found family clustering in 7 families of 219 male and female pro-

positi and more frequently among families of female than male patients In our study clustering of deaths due to IHD was found in 8 7% and 4 7% of the 150 families of propositi and controls respectively and a striking preponderance among relatives of female propositi in both groups

The possible inheritance of IHD

Many of the authors cited above have pointed out that IHD might be influenced by a number of genes and thus be an example of a polygenic inherited trait that is affected by the environment

In the present study the IHD deaths indicate an increased risk to close relatives of coronary patients over controls and over the general population which suggests some common cause between relatives with respect to the disease This could be due to either common genes or common environment It is obvious from the present study population that IHD risk is not inherited in a simple manner and that a sex linkage is unlikely but that a delayed manifestation of the genes in females rather shows a partial sex limitation which could be a hormonal effect However the relatives of female patients show a substantially more pronounced increase in risk of death from IHD than those of male patients Among fathers and brothers of female patients this increase may be as high as 7 fold compared with the general population whereas it is only 2–3 fold among those of male patients This could indicate an additive polygenic inheritance of the disease where a heavy load of affecting genes is needed to cause IHD deaths in females the apparently more resistant sex It is also shown that the risk to first degree relatives is 3 fold above the expected value or twice as high as that to the second degree relatives which is typical for genetic factors that decrease as the relationship become more remote

According to Falconer (4) an estimate of heritability can be determined to show the correlation of liability between relatives In the present study the heritability was calculated for male and female propositi of first degree relatives for both male and female relatives in relation to controls as well as to the general population The heritability was found to be 20%±10 as roughly combined results of males and 30–35%±15 among female propositi in the first generation which gives a much lower heritability estimate than reported by others i e 60% risk to males and 70% to females (17) and 80% to males (13) Our figures are comparable with the likelihood of heritability found for peptic ulcer (4) It must be borne in mind that a familial aggregation of this kind with a high susceptibility for father son and uncle might also be an indication of a shared environment or a combination with a genetic factor as there is a tendency for traditions operating within families such as common dietary habits often to be passed down mainly on the female side but to affect all family members This might to the same degree affect males in the first as well as the second generation but could pass unobserved among females due to the delayed onset among the latter

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REFERENCES

1 Cederlof R Friberg L & Jonsson E Hereditary factors and angina pectoris Arch Environ Health 14 397 1967
2 Deutscher S Ostander L D & Epstein F H Familial factors in premature coronary heart disease—a preliminary report from the Tecumseh community study Am J Epidemiol 91 3 1970
3 de Faire U Ischaemic heart disease in death discordant twins Acta Med Scand (Suppl) 568 96 1974
4 Falconer D S The inheritance of liability to certain diseases estimated from the incidence among relatives Ann Hum Genet 29 51 1965
5 Gertler M M & White P D Coronary heart disease in young adults a multidisciplinary study Harvard University Press Cambridge Mass 1954
6 Harvald B & Hauge M Hereditary factors elucidated by twin studies *In Genetics and epidemiology* of chronic diseases pp 61–76 US Public Health Service Publ no 1103 Washington D C 1963
7 Coronary occlusion in twins Acta Genet Med Gemellol 19 248 1970
8 Liljefors I Coronary heart disease in male twins Acta Med Scand (Suppl) 511 78 1970
9 Palmer A J & Blacket R B Genetics of coronary heart disease Singapore Med J 14 3 1973
10 Phillips R Lilienfeld A Diamond E I & Kagan A Frequency of coronary heart disease and cerebrovascular accidents in parents and sons of coronary heart disease index cases and controls Am J Epidemiol 100 2 1974
11 Population and Vital Statistics 1951–60 pp 52 72 74 Stat Bureau of Iceland Reykjavik 1963
12 — 1961–70 pp 13 35 49 55 Reykjavik 1975
13 Rissanen A M & Nikkila E A Coronary artery disease and its risk factors in families of young men

with angina pectoris and in controls Br Heart J 39 875 1977

14 Rose G Familial patterns in ischaemic heart disease Br J Prev Soc Med 18 75 1964

15 The epidemiology of coronary heart disease in Europe In Publ Health in Europe 2 p 48 WHO Copenhagen 1973

16 Shanoff H M Little A Murphy E A & Rykert E Studies of male survivors of myocardial infarction due to essential arteriosclerosis Can Med Ass J 84 519 1961

17 Slack J & Ewans K A The increased risk of death from ischaemic heart disease in first degree relatives of 121 men and 96 women with ischaemic heart disease J Med Genet 3 239, 1966

18 Stat Bureau of Iceland personal communication

19 Thomas C B & Cohen B H The familial occurrence of hypertension and coronary artery disease with observations concerning obesity and diabetes Ann Intern Med 42 90 1955

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Platelet Survival and Platelet Production in Acute Myocardial Infarction

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ABSTRACT Thrombokinetic studies were carried out on 26 consecutive patients with myocardial infarction (MI) admitted to a coronary care unit in the acute stage during a two-month period The results were compared with those of an age-matched control group In the MI patients platelet mean life-span was 5 0±0 3 days and significantly shorter ($p<0\ 01$) than in the controls (6 4±0 4 days) The mean platelet production rate for the patients with MI was significantly higher ($p<0\ 005$) than for the controls On the basis of results reported by others as well as the present data, it is suggested that during the acute phase of MI there is no additional measurable reduction in platelet survival above that observed in chronic coronary artery disease

Key words myocardial infarction platelet kinetics

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Platelets contribute to the development of occlusive vascular disease (4 6 7 14) Several reports on platelet survival in coronary artery disease (CAD) have been published (1 10 12 13 15 16 17) and shortened platelet survival was reported in most of them Little information is however available concerning platelet kinetics during an acute attack of myocardial infarction (MI) The present study provides data on platelet survival and platelet production in patients with recent MI

STUDY POPULATION

Platelet survival studies were performed on consecutive patients who were admitted to a coronary care unit between 8 p m and 8 a m during a two month period In formed consent to undertake the investigations was obtained from all

The criteria for entering the study were proved or strongly suspected recent MI in the absence of disorders known to be associated with shortened platelet survival (see below) The diagnosis of MI was based upon a history of chest pain presence of typical ECG changes and characteristic elevations of aspartate aminotransferase and lactate dehydrogenase activities in serum The platelet survival study was started in every case within 72 hours after the onset of symptoms supposedly attributable to MI Patients with hematological disorders alcoholism diabetes mellitus renal disease and malignancies did not enter the study None of the cases examined was excluded from the study None of the patients received anticoagulant therapy prior to or during the investigation

Out of a total of 30 patients (27 males and 3 females) (Table I) 26 (23 males and 3 females mean age 60 years range 43–71) had MI according to the criteria given above The initial suspicion of MI could not be confirmed in 4 patients

The control group consisted of 16 male subjects with a mean age of 57 years (range 44–78) (Table I) Eight of them were completely healthy volunteers selected from a population study and 8 were patients admitted to hospital because of mild or moderate cardiovascular disorders (CVD)

METHODS

All studies were carried out with autologous platelets The labelling technique has been described in detail elsewhere (9) Only a brief summary of the procedure is given below

Whole blood (approximately 125 ml) was collected by gravity into a plastic bag containing 20 ml of acid ACD (3) and centrifuged at 260 *g* The platelet rich plasma was then transferred to another bag and centrifuged at 1 600 g to provide a platelet button which was resuspended in 10 ml of plasma and incubated with 600 μCi of ^{51}Cr in the form of sodium chromate Thereafter the incubated platelets were washed with platelet poor plasma and finally injected into the subject Blood sampling was made in duplicate 1 hour after the infusion of labelled platelets and

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Abbreviations MI=myocardial infarction CAD–coronary artery disease CVD–cardiovascular disorders MLS= mean life span PBR=platelet-bound radioactivity

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with angina pectoris and in controls Br Heart J 39 875 1977
14 Rose G Familial patterns in ischaemic heart disease Br J Prev Soc Med 18 75 1964
15 The epidemiology of coronary heart disease in Europe In Publ Health in Europe 2 p 48 WHO Copenhagen 1973
16 Shanoff H M Little A Murphy E A & Rykert E Studies of male survivors of myocardial infarction due to "essential arteriosclerosis Can Med Ass J 84 519 1961
17 Slack J & Ewans K A The increased risk of death from ischaemic heart disease in first degree relatives of 121 men and 96 women with ischaemic heart disease J Med Genet 3 239 1966
18 Stat Bureau of Iceland personal communication
19 Thomas C B & Cohen B H The familial occurrence of hypertension and coronary artery disease with observations concerning obesity and diabetes Ann Intern Med 42 90 1955

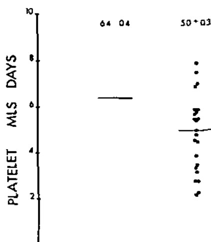


Fig 1 Platelet MLS (mean±S E) in 16 control subjects (O) and 26 patients with recent MI (●)

where 90%/recovery % is the correction for the splenic pooling of platelets (9) The counting of platelets was performed with a Coulter counter Model F as previously described (9)

Unless otherwise stated mean values ±standard error of the mean (S E) are reported Mean values were tested according to Student s *t* test The difference between mean values was considered statistically significant if $p<0.05$

RESULTS

In the following only the results for patients with confirmed MI and control subjects are reported The results for the 4 patients in whom MI was not established are given in Table I

The mean venous platelet count was $181\pm6\times10^9$/l in the controls and $187\pm9\times10^9$/l in the MI patients (Table I)

Recovery of infused platelets The mean platelet recovery was equal in the two groups studied (59 ± 3 and 60 ± 3% respectively) Four MI cases had values below the range of the controls (Table I)

Platelet mean life span There was a considerable scatter of individual values for platelet MLS in the controls as well as in the patients with MI (Fig 1 Table I) For the 8 completely healthy volunteers the average platelet MLS was 6.6 ± 0.5 days the corresponding value for the 8 control subjects with mild or moderate CVD being 6.1 ± 0.6 The mean platelet MLS for the MI patients (5.0 ± 0.3 days) was significantly lower ($p<0.01$) than for the 16 controls (6.4 ± 0.4 days) While the mean for the 8 healthy volunteers differed significantly from the mean for the MI patients there was no statistical difference between the 8 control subjects with CVD and the MI patients ($t=1.53$) Seven MI patients had platelet MLS values below the range of the controls

Platelet production rate the mean as well as the range in the patients with MI exceeded the mean and range for the controls (Table I) The mean rate was $22\pm1\times10^{10}$ platelets per day in the controls and $31\pm2\times10^{10}$ in the MI patients The difference between these means was highly significant ($p<0.005$)

DISCUSSION

Ritchie and Harker (13) and Steele et al (15 16) recently published their results of platelet survival studies carried out on a large number of patients with CAD In these series every subject had arteriographic evidence of CAD and 53–69% had shortened platelet survival Compared to the controls the mean platelet survival for patients with CAD was reduced by 24% (13) 22% (16) and 14% (15) respectively In addition Steele et al (16) reported on the results of platelet survival studies on 8 men with MI and normal coronary arteriogram All of them had had an acute transmural infarction 6–20 months before the study Platelet survival was shortened in all 8 men and compared to 8 age matched normal men their mean platelet survival was reduced by 35%

In acute MI it might be expected that platelet consumption is beyond that observed in CAD Abrahamsen (1) published results of platelet survival studies on 15 patients with recent MI On admission to hospital his patients were allocated at random to one of two groups one of which was started on phenylindandione or dicoumarol while the other received no anticoagulants The experiments were started 2–4 days after admission Anticoagulant therapy had no effect on platelet survival which was reduced in both groups

In the present work 7 (27%) out of the 26 patients with MI had platelet survival below the range of an age matched control group For the MI patients the mean value for platelet MLS was 22% shorter than for the controls Platelet recovery representing the splenic platelet pool did not differ between MI patients and controls

The mean platelet production in the patients with MI was 1 4 times higher than in the controls the

difference being statistically significant ($p<0.005$) A similar increase in platelet production has consistently been reported by workers who have studied patients with CAD as well as patients with other arteriosclerotic disorders (1 2 5 8 9 10) It might well be that this finding reflects a participation by platelets in the development of atherosclerosis and its complications However it could also be speculated that the increased turnover of platelets is a phenomenon secondary to atheromatosis

On the basis of the aforementioned it could be concluded that patients with CAD frequently have shortened platelet survival In the acute phase of an MI however there does not seem to be any further measurable reduction in platelet survival

ACKNOWLEDGEMENT

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REFERENCES

1 Abrahamsen A F Platelet survival studies in man With special reference to thrombosis and atherosclerosis Scand J Haematol (Suppl) 3 1968
2 Abrahamsen A F Eika C Godal H C & Lorentsen E Effect of acetylsalicylic acid and dipyridamole on platelet survival and aggregation in patients with atherosclerosis obliterans Scand J Haematol 13 241 1974
3 Aster R H & Jandl J H Platelet sequestration in man I Methods J Clin Invest 43 843 1964
4 Didisheim P & Fuster V Actions and clinical status of platelet suppressive agents Semin Haematol 15 55 1978
5 Gilbert J B & Mustard J F Some effects of Atromid on platelet economy and blood coagulation in man J Atheroscler Res 3 623 1963
6 Harker L A Inhibitors of platelet function in the prevention of arterial thrombosis Ser Haematol 8 105 1976
7 — The kinetics of platelet production and destruction in man Clin Haematol 6 671 1977
8 Harker L A & Slichter S J Platelet and fibrinogen consumption in man N Engl J Med 287 999 1972
9 Kutti J & Weinfeld A Platelet survival in man Scand J Haematol 8 336 1971
10 Murphy E A & Mustard J F Coagulation tests and platelet economy in atherosclerotic and control subjects Circulation 25 114 1962
11 Nadler S B Hidalgo J U & Block T Prediction of blood volume in normal human adults Surgery 51 224 1962
12 O Neil B & Firkin B Platelet survival studies in coagulation disorders thrombocythemia and conditions associated with atherosclerosis J Lab Clin Med 64 188 1964
13 Ritchie J L & Harker L A Platelet and fibrinogen survival in coronary atherosclerosis Response to medical and surgical therapy Am J Cardiol 39 595 1977
14 Sahud M A The platelet and coronary artery disease Ser Haematol 8 125 1976
15 Steele P Battock D & Genton E Effects of clofibrate and sulfinpyrazone on platelet survival time in coronary artery disease Circulation 52 473 1975
16 Steele P Rainwater J & Vogel R Abnormal platelet survival time in men with myocardial infarction and normal coronary arteriogram Am J Cardiol 41 60 1978
17 Steele P P Weily H S Davies H & Genton E Platelet function studies in coronary artery disease Circulation 48 1194 1973

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Peripheral Hemodynamics in Assisted Circulation with Intra-Aortic Balloon Pumping in Patients with Cardiogenic Shock

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ABSTRACT Seven patients treated for cardiogenic shock were studied with and without intra aortic balloon pumping (IABP) Calf and forearm blood flows were determined with a Dohn plethysmograph and arterial pressures were registered intra-arterially and in the great toe and thumb with the cuff method During IABP, an augmented flow was registered in the arms and legs and accurate arterial BPs could also be determined from the extremities The findings demonstrate a beneficial effect of IABP on peripheral flow especially in patients who could be weaned off the pump

Key words acute myocardial infarction invasive and non-invasive technique assisted circulation intra aortic balloon pumping cardiogenic shock peripheral hemodynamics

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Cardiogenic shock has been treated with mechanical circulatory assistance during the last decade by means of a variety of methods both experimentally and clinically One method however intra aortic balloon pumping (IABP) has been used more widely in the clinical situation mainly for treatment of shock due to acute myocardial infarction (AMI) and postoperatively after open heart surgery The aim of the method is threefold 1) To augment aortic diastolic pressure thereby increasing the coronary perfusion pressure and myocardial nutrition 2) To reduce the aortic systolic pressure thereby decreasing the afterload and oxygen consumption of the heart 3) To increase the perfusion of peripheral tissues The effect of IABP on central hemodynamics has been investigated thoroughly in several clinical studies (1 2 8) but the effect on the perfusion of peripheral tissues has been reported only in terms such as disappearance of cyanosis and coldness of the skin recovered consciousness and increased urine flow—all indirect evidence of elevated flow to these tissues

The present investigation was undertaken with the aim of studying the effect of IABP on the peripheral circulation by recording flow and pressures in the arms and legs

PATIENTS AND METHODS

Seven patients six men and one woman treated with IABP for cardiogenic shock due to AMI were studied (Table I) Their age varied between 54 and 70 years Two patients had a history of previous myocardial infarction The AMI involved the anterior wall in all cases and was according to the ECG extensive in three The time in shock varied between 5 and 70 hours Shock was defined as a palpatory systolic blood pressure (BP) below 90 mmHg for more than half an hour in association with at least three of the following findings 1) Signs of reduced cerebral circulation such as mental confusion or unconsciousness 2) Signs of reduced peripheral circulation such as cold skin 3) Signs of reduced renal circulation with a urine flow less than 20 ml/hour 4) Peripheral or general cyanosis 5) Metabolic acidosis

Patients fulfilling the above criteria of shock were given medical treatment as described in detail earlier (8) and not including vasopressor drugs Those still in shock despite this therapy were given IABP with a three segment balloon (the AVCO system) No vasodilator drugs were added during IABP

The balloon catheter was inserted into the femoral artery after local anesthesia advanced to the descending part of the thoracic aorta with the tip just distal to the left subclavian artery (Fig 1) and then connected to the driving unit The balloon was inflated with helium during

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Abbreviations IABP=intra aortic balloon pumping AMI=acute myocardial infarction BP=blood pressure

Table I Clinical findings and outcome
A=anterior L=lateral D=diaphragmatic

Pat no	Age (y)	Previous infarction	Site of infarction	Duration of shock (h)	Duration of IABP (h)	Out of shock	Weaned off IABP	Hospital survival
1	54	–	AL	15	36	+	+	+
2	70	+	A	26	58	+	+	–
3	70	–	ALD	11	318	+	–	–
4	60	–	AL	27	12	–	–	–
5	58	–	A	5	28	+	+	–
6	60	–	ALD	14	19	+	–	–
7	60	+	ALD	70	85	+	–	–

diastole and rapidly deflated just prior to systole The onset of inflation/deflation was synchronized with the aortic pressure curve (Fig 2) Balloon inflation caused an increased diastolic pressure and balloon deflation a reduced systolic pressure of the aorta (Fig 2)

A teflon catheter (o d 1 2 mm) was inserted percutaneously into the brachial artery in one arm and advanced to the aorta Intra arterial pressures were registered with transducers of the variable capacitance type (EMT 34 Siemens Elema) and recorded on a Mingograph

The blood flow through the calf (in 7 subjects) and the forearm (in 5 subjects) was determined with a modified Dohn plethysmograph (4) The changes in volume during proximal venous occlusion were measured with an air filled cuff of latex rubber placed around the thickest part of the extremity While the blood flow was being measured the flow to the foot and hand was closed off by another cuff The venous occlusion pressure was 60 mmHg and the pressure in the plethysmograph cuff about 3 mmHg The pressure changes in the rubber plethysmograph cuff induced by venous occlusion were measured with a pressure transducer (EMT 31 Siemens Elema) and recorded on a Mingograph Calibration was undertaken by inflating 2 ml air into the cuff The mean value of five measurements was calculated on each occasion

For distal BP measurements a small cuff (width 24 mm) was placed around the base of the great toe and the thumb A mercury insilastic strain gauge was placed distally to the cuff balanced on a Wheatstone bridge and connected to a recording unit (Mingograph) The pressure was recorded as described by Gundersen (5)

The measurements were performed during IABP as well as during a period of non assisted circulation when the pump had been shut off for at least 15 min The recordings were made according to a randomized system starting during assisted circulation in four patients and during pump off in three All recordings were made 10–24 hours after initiation of the IABP The catheter free arm and the leg without balloon catheter were always chosen for the measurements of peripheral hemodynamics

Standard statistical methods were employed for analysing the results using the paired t test when applicable Data are presented as mean±S E



Fig 1 Bedside X ray of the chest showing the inflated balloon catheter in the descending part of the aorta

RESULTS

Clinical findings and outcome are shown in Table I The duration of IABP varied between 12 and 318 hours Shock symptoms were reversed in all patients but one and three could be weaned off IABP and have the balloon extracted Two of these (pats 2 and 5) however had a fatal reinfarction after 16 and 19 days respectively Patient 1 was long term survivor Four patients were pump-dependent Patient 3 had a large ventricular septal defect which was not closed surgically Extensive AMI involving more than 65% of the left ventricle was evident at autopsy in patients 4 6 and 7

The aortic systolic pressure was 91±7 mmHg without assisted circulation and a peak diastolic

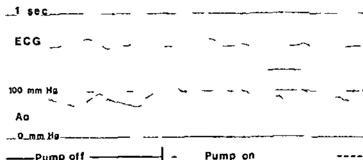


Fig 2 Aortic pressure (*Ao*) with and without IABP Note the increased diastolic and decreased systolic pressure when the pump is switched on

pressure of 99±6 mmHg was recorded during IABP (Table II)

The cuff systolic pressure in the thumb without assisted circulation 77±11 mmHg did not differ significantly from the intra arterial pressure in the aorta whereas the pressure in the great toe 56±9 mmHg was 38% lower ($p<0.05$) With the pump on the peak diastolic pressure in the aorta was somewhat higher than in the thumb 99±6 vs 82+8 mmHg although the difference was not significant The peak diastolic pressure in the toe 68±6 mmHg was 30% lower than the intra arterial ($p<0.05$) (Table II)

As shown in Fig 3 forearm blood flow without assisted circulation was 1 74±0 22 ml min⁻¹ 100 g⁻¹ tissue and increased significantly during IABP to 1 90±0 25 ($p<0.05$) The calf blood flow showed a somewhat higher increase during IABP (1 05±0 06 vs 1 56±0 19 ml min⁻¹ 100 g⁻¹ $p<0.05$)

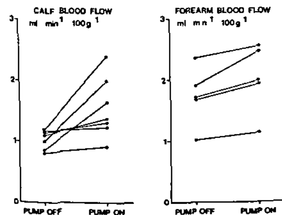


Fig 3 Calf and forearm blood flow with and without assisted circulation

DISCUSSION

One important aim of assisted circulation is to increase the perfusion in peripheral tissue and the main finding in this study is that blood flow in the arms and legs could be augmented during IABP (Fig 3) Without assisted circulation the flow in the extremities was low approximately 50% of the normal value in healthy subjects (9) This decrease is about the same as that reported for cardiac output (8) The increase in the peripheral flow during IABP showed great interindividual differences a finding that applies also to the central flows (8) Patient 1 was the only long term survivor and had the highest flow without assisted circulation and an increase of more than 50% in leg blood flow when on IABP No difference was found in leg blood flow without assisted circulation between patients who could or could not be weaned off IABP The increase in flow during IABP however was twice as marked in the CCU survivors than in the non survivors and these results agree with values for central flow reported previously (3) In this study an increase of 40% in cardiac output was found in balloon independent

Table II *Systolic pressures without (pump off) and peak diastolic pressures with (pump on) assisted circulation (mean±S E)*

	Aorta	Thumb	Great toe
Systolic BP (mmHg)			
Pump off	91+7	77±11	56±9
Peak diastolic BP (mmHg)			
Pump on	99±6	82± 8	68±6

patients after 12 hours pumping against only 3 % in balloon-dependent patients

The peak pressures achieved in the thumb were somewhat but not significantly lower than the intra aortic registration indicating a pressure of the same magnitude in the small finger arteries as in the aorta The pressure in the great toe was significantly lower than the intra arterial registration The difference of about 30 mmHg is however the same as expected in healthy subjects of the same age and can be explained by hydrostatic and local factors (5) The present study shows that the peak diastolic cuff pressure during assisted circulation can be measured with the same accuracy as the systolic cuff pressure without IABP and that the toe pressure shows the same percentage of the intra aortic pressure with as well as without assisted circulation

In conclusion the data from this study demonstrate a beneficial effect of IABP on peripheral circulation with an increased calf blood flow The flow increased more in patients who could be weaned off the pump than in pump dependent patients indicating that the flow measurements may be useful in identifying balloon-dependent patients already during the first 24 hours Moreover BP can be accurately measured with the strain gauge technique although this method does not give any additional information compared to the necessary in aortic registration

REFERENCES

1 Bardet J Masquet C Kahn J-C Gourgon R Bourdarias J P Mathivat A & Bouvrain Y Clinical and hemodynamic results of intra aortic balloon counterpulsation and surgery for cardiogenic shock Am Heart J 93 280 1977

2 Dunkman W B Leinbach R C Buckley M J Mundth E D Kantrowitz A R Austen W G & Sanders C A Clinical and hemodynamic results of intra aortic balloon pumping and surgery for cardiogenic shock Circulation 46 465 1972

3 Ehrich D Biddle T Kronenberg M & Yu P The hemodynamic response to intra aortic balloon counterpulsation in patients with cardiogenic shock complicating acute myocardial infarction Am Heart J 93 274 1977

4 Graf K & Westersten A Untersuchungen uber Eigenschaften und Moglichkeiten eines flexiblen Extremitaten Plethysmographen Acta Physiol Scand 46 1 1959

5 Gundersen J Segmental measurements of systolic blood pressure in the extremities including the thumb and the great toe Acta Chir Scand (Suppl) 426 15 1972

6 Leinbach R C Buckley M J Austen N G Petschek H E Kantrowitz A R & Sanders C A Effects of intra aortic balloon pumping on coronary flow and metabolism in man Circulation (Suppl) 43 1971

7 Mueller H Ayres S M Conklin E F Giannelli S Jr Mazzara J T Gruce W T & Nealon T F Jr The effects of intra aortic counterpulsation on cardiac performance and metabolism in shock associated with acute myocardial infarction J Clin Invest 50 1885 1971

8 Nyquist O Shock complicating acute myocardial infarction Acta Med Scand (Suppl) 536 1972

9 Siggaard Andersen J & Petersen Bonde F Venous occlusion plethysmography and ^{133}Xe muscle clearance measured simultaneously on the calf in normal subjects Scand J Clin Lab Invest 19 106 1967

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Verapamil-Induced Ventricular Regularity in Atrial Fibrillation

Effects of Exercise, Isoproterenol, Atropine and Conversion to Sinus Rhythm

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ABSTRACT. The effects of physical exercise, isoproterenol and atropine upon the ventricular rate and regularity were studied in 12 patients with atrial fibrillation (AF) during verapamil induced regular ventricular rhythm. Physical exercise, isoproterenol and atropine all caused a gradual loss of ventricular regularity during AF. Verapamil caused a reduction of exercise heart rate during AF and, to a minor extent, even during sinus rhythm. The regular ventricular rhythm induced by verapamil during AF is interpreted as a total AV nodal block with nodal escape rhythm. Possible clinical benefits of this rhythm are discussed.

Key words: atrial fibrillation, ventricular regularity, verapamil.

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We have previously demonstrated that verapamil given by mouth to patients with atrial fibrillation (AF) will produce a regular ventricular rhythm even though AF persists (5). This effect is achieved in all patients provided the dose is sufficiently high. Thus 6 out of 10 patients on chronic digitalis treatment developed regular ventricular rhythm after 240 mg of verapamil by mouth and all 10 showed this effect after a stepwise increase of the dose to 400 mg.

The present paper aims to illustrate any changes in the ventricular rate and regularity when patients in AF with verapamil induced regular ventricular rhythm are exposed to physiological and pharmacological factors known to influence atrioventricular (AV) conduction. A second aim is to compare changes in heart rate induced by these factors when the patients are in sinus rhythm (SR) and AF respectively, and under the influence of high doses of verapamil.

PATIENTS AND METHODS

Seventeen patients with chronic AF were recruited to the study. They were all admitted to our department for DC conversion of AF. All patients were taking digitalis regularly but none was taking β blockers. The procedure was explained and the patients' informed consent was obtained. The design of the study was approved by the Ethical Committee of the hospital.

Protocol. 1. ECG after 30 min rest, fasting phase. 2. Exercise test. 3. Verapamil 240 mg by mouth (3 tablets of 80 mg), thereafter hourly ECG recordings for up to 4 hours. 4. Exercise test when ventricular regularity appears. 5. Isoproterenol test one hour after exercise test, if ventricular regularity persists. 6. Atropine test one hour after isoproterenol test, if ventricular regularity persists. 7. DC conversion 2–4 days later. If SR was achieved, no change in therapy was undertaken. 8. If the patient was still in SR 2–7 days after DC conversion, identical repetition of procedures 1–6. Special care was taken to perform the exercise, isoproterenol and atropine tests at the same times after verapamil intake as during AF.

ECG tracings were recorded for 1 min at a paper speed of 100 mm/sec. Blood pressure (BP) measurement was always performed immediately after all ECG recordings using the sphygmomanometer method.

Mean values of RR intervals were calculated from all ECG recordings by measuring all individual RR intervals during the 1 min recording. The standard deviation (SD) and variation coefficient (VC) of RR intervals were also calculated for each ECG recording.

An *exercise test* was performed on a bicycle ergometer in the seated position. Exercise was started at 20 W and the ECG was recorded between the 2nd and 3rd min on this load. The load was then increased by steps of 20 W with ECG recording during the last minute on each load. In the initial test, when the patient was in AF and not on verapamil, the exercise load was increased to a level such that the patient experienced subjective symptoms and felt that he was unable to perform further exercise. In the subsequent exercise tests, the load was increased only to the maximum level of the initial exercise test.

Abbreviations: AF = atrial fibrillation, SR = sinus rhythm, AV = atrioventricular, BP = blood pressure, SD = standard deviation, VC = variation coeffi-

Table I *Clinical details of the patients who showed ventricular regularity after 240 mg verapamil*
MI=myocardial infarction MS=mitral stenosis MIC=mitral incompetence

Pat no	Age (y)	Sex	Diagnosis	Duration of AF	NYHA functional group	Total heart volume/m² BSA (ml)	Medication
1	39	♂	Lone AF	5 y	I	930/500	Digoxin dicoumarol
2	52	♂	Lone AF	3 weeks	I	1 000/540	Digoxin
3	61	♂	Lone AF	6 mo	II	1 150/590	Digoxin dicoumarol
4	65	♂	Lone AF	>4 mo	II	1 000/480	Digoxin dicoumarol
5	56	♂	Lone AF	6 mo	II	1 010/460	Digoxin dicoumarol
6	64	♂	MI	1½ y	I–II	1 100/420	Digoxin dicoumarol
7	65	♂	MS+MIC	1 y	II–III	1 170/580	Digoxin dicoumarol furosemide+ potassium chloride
8	47	♂	Lone AF	6 mo	II	880/450	Digoxin dicoumarol
9	59	♂	Lone AF	>2 mo	I	830/460	Digoxin dicoumarol
10	60	♀	Lone AF	6 mo	III	1 100/640	Digoxin dicoumarol verapamil
11	50	♂	Lone AF	>6 mo	II	780/440	Digoxin dicoumarol
12	52	♂	Lone AF	6 mo	II	800/390	Digoxin furosemide+ potassium chloride dicoumarol

The *atropine test* included an ECG recording 1 min before and up to 10 min after a bolus dose of 0 5 mg of atropine i v The ECG after atropine was analysed over the 1 min period with the highest observed heart rate

The isoproterenol test included an ECG recording 1 min before and up to 10 min after a bolus dose of 2 µg of isoproterenol i v The ECG after isoproterenol was analysed over the 1 min period with the highest observed heart rate

RESULTS

trial fibrillation

Of the 17 patients who entered the study 12 developed ventricular regularity after 240 mg of verapamil by mouth As a criterion of ventricular regularity we required VC of RR interval of ≤12% (5) Clinical data on these 12 patients are given in Table

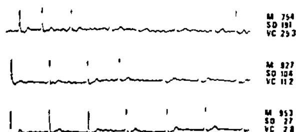


Fig 1 Precordial differential ECG with mean (*M*) standard deviation (*SD*) and variation coefficient (*VC*) of RR intervals from a patient in AF Top before verapamil Middle under slight influence of verapamil Bottom when verapamil has induced pronounced ventricular regularity

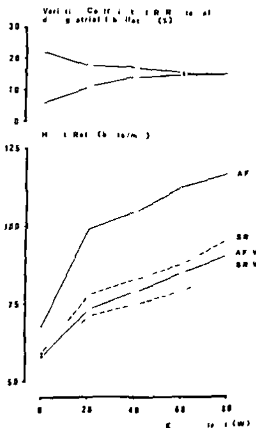


Fig 2 Effect of different exercise levels upon ventricular regularity and rate in cases 1–5 during AF as well as during SR with and without the influence of 240 mg of verapamil (V) by mouth (mean values of individual observations or calculations)

Table II *Effect of exercise upon ventricular rate and regularity with and without verapamil during AF*

Pat no		RR interval (msec)													
		Before verapamil							With verapamil						
		Rest	20 W	40 W	60 W	80 W	100 W	120 W	Rest	20 W	40 W	60 W	80 W	100 W	120 W
1	*M*	883	729	673	647	584	–	–	761	722	685	631	604	–	–
	SD	181	134	106	89	114	–	–	123	29	25	34	38	–	–
	VC	20	18	16	14	20	–	–	3	4	4	5	6	–	–
2	*M*	1 078	708	669	623	587	555	485	1 212	938	867	799	743	684	631
	SD	242	130	124	100	80	91	82	32	120	144	146	147	123	110
	VC	22	18	19	16	14	16	17	3	13	17	18	20	18	18
3	*M*	1 004	683	662	564	510	450	428	1 364	905	822	760	681	614	550
	SD	340	151	151	115	93	67	62	99	158	157	138	136	109	82
	VC	34	22	23	20	18	15	14	7	17	19	18	20	18	15
4	*M*	706	497	480	441	418	388	–	1 010	806	744	686	645	594	–
	SD	130	78	82	71	63	55	–	112	153	149	110	103	78	–
	VC	18	16	17	16	15	14	–	11	19	20	16	16	13	–
5	*M*	455	402	386	388	368	–	–	793	731	690	653	607	–	–
	SD	98	76	77	60	59	–	–	18	85	83	91	80	–	–
	VC	22	19	20	16	16	–	–	2	12	12	14	13	–	–
6	*M*	940	726	659	602	497	–	–	1 163	901	801	731	648	–	–
	SD	235	131	124	99	89	–	–	66	118	128	116	86	–	–
	VC	25	18	19	16	18	–	–	6	13	16	16	13	–	–
7	*M*	721	452	435	406	374	–	–	1 003	680	640	604	555	–	–
	SD	139	69	64	58	47	–	–	55	99	122	111	105	–	–
	VC	19	15	15	14	12	–	–	5	15	19	18	19	–	–
8	*M*	802	627	575	505	437	395	348	1 071	854	790	700	623	556	513
	SD	200	146	112	89	64	62	53	31	72	104	134	124	116	107
	VC	25	23	19	18	15	16	15	3	8	13	19	20	21	21
9	*M*	802	664	632	579	522	480	416	1 043	810	711	656	601	562	518
	SD	110	85	87	80	69	56	35	106	36	42	49	54	49	54
	VC	14	13	14	14	13	12	9	10	4	6	7	9	9	10
10	*M*	407	351	349	345	–	–	–	711	559	462	429	–	–	–
	SD	69	49	44	36	–	–	–	44	75	82	79	–	–	–
	VC	17	14	13	10	–	–	–	6	13	18	18	–	–	–
11	*M*	832	676	653	586	548	485	415	1 177	933	849	762	679	611	517
	SD	239	143	129	128	96	99	87	100	155	161	135	143	116	100
	VC	29	21	20	22	18	20	21	8	17	19	18	21	19	19
Mean in column	*M*	785	592	561	517	485	459	418	1 028	804	733	674	639	604	546
	SD	180	108	100	84	77	72	64	71	100	109	104	102	99	91
	VC	22.3	17.9	17.7	16.0	15.9	15.5	15.2	5.8	12.2	14.8	15.2	15.7	16.3	16.6

I All patients had a BP drop of 10–20 mmHg systolic during the first 2 hours after verapamil. In no patient did the systolic BP fall below 100 mmHg. Fig. 1 depicts the ECG and calculated values of mean RR and VC of RR at different degrees of ventricular regularity.

Effect of verapamil at rest and during exercise (Table II). Eleven out of 12 patients performed the exercise test. Patient 12 was unable to exercise owing to a knee joint disease. Mean RR intervals with SD and VC at rest and at each exercise level during AF are given in Table II. The VC of RR intervals ranged between 14 and 34% at rest before verapamil was given. Exercise induced a slight decrease in this value in most patients, indicating a slightly more regular ventricular rhythm during exercise than under resting conditions. This initial regularity persisted during the entire exercise test in patients 1 and 9, but was lost even on low exercise loads in most of the others. The mean VC values indicate a gradual loss of verapamil induced ventricular regularity with increasing exercise loads until the same RR variability is reached as during the initial exercise test (Fig. 2). All patients were able to

Table III *Effect of isoproterenol and atropine upon verapamil induced ventricular rate and regularity during AF*

NR=not recorded

Pat no		RR interval (msec)			
		Before isoproterenol	With isoproterenol	Before atropine	With atropine
1	M	946	702	1 105	846
	SD	43	67	94	89
	VC	5	10	9	11
2	M	1 245	1 116	1 270	1 209
	SD	21	52	25	77
	VC	2	5	2	6
3	M	1 254	1 019	1 279	966
	SD	35	86	65	101
	VC	3	8	5	10
6	M	1 265	1 078	1 340	1 075
	SD	19	98	62	41
	VC	2	9	5	4
7	M	1 046	912	1 174	944
	SD	25	25	27	9
	VC	2	3	2	1
8	M	1 122	926	NR	
	SD	36	139	–	–
	VC	3	15		
9	M	1 090	857	NR	
	SD	57	58	–	–
	VC	5	7		
10	M	769	622	NR	
	SD	50	110	–	–
	VC	6	18		
12	M	670	662	842	747
	SD	22	16	13	38
	VC	3	2	1	5
Mean in column	M	1 045	877	1 168	965
	SD	34	72	48	59
	VC	3 4	8 6	4 0	6 2

reach the same exercise loads as before verapamil administration

Heart rate decreased after verapamil in all cases but one (pat 1) at rest and during exercise as indicated by an increase in the RR interval Patient 1 showed only a minor effect on heart rate although a marked ventricular regularity had developed

Effects of isoproterenol and atropine after verapamil during AF (Table III) Verapamil induced a ventricular regularity which persisted for at least one hour after the exercise test in 9 of the 12 patients One hour later only 6 patients still exhibited ventricular regularity Thus 9 patients were included in the isoproterenol study and 6 in the atropine study

Isoproterenol in a dose of 2 μg induced an increase in heart rate and a slightly less regular ventricular rate in all patients However only 2 of the 9 patients exhibited an increase in their RR VC to over 12% and according to our definition 7 of the 9 patients thus remained in ventricular regularity

Atropine in a dose of 0 5 mg raised the heart rate in all 6 patients but all of them maintained an RR VC of less than 12% Slight increases in RR VC were however observed in 4 of the 6 patients

Sinus rhythm

DC conversion resulted in SR of sufficiently long duration to allow studies of the effect of verapamil in 5 of the 12 patients

Effect of verapamil at rest and during exercise (Table IV) All patients were able to reach the same

Table IV *Effect of exercise upon ventricular rate and regularity with and without verapamil during SR*

Pat no		RR interval (msec) Before verapamil Rest	20 W	40 W	60 W	80 W	100 W	120 W	With verapamil Rest	20 W	40 W	60 W	80 W	100 W	120 W
1	*M*	833	742	700	650	594	–	–	955	782	733	691	623	–	–
	SD	80	42	19	13	14	–	–	28	34	14	24	24	–	–
	VC	10	6	3	2	2	–	–	3	4	2	3	4	–	–
2	*M*	1 047	712	669	646	602	570	537	1 007	745	708	679	636	601	551
	SD	61	60	41	28	18	12	13	69	12	35	42	27	32	26
	VC	6	8	6	4	3	2	2	7	2	5	6	4	5	5
3	*M*	1 365	825	776	713	642	591	542	1 404	984	916	854	750	675	636
	SD	32	47	14	27	16	9	40	92	64	54	36	19	7	7
	VC	2	6	2	4	2	2	7	7	7	6	4	2	1	1
4	*M*	1 007	826	782	728	664	608	–	1 183	938	921	854	792	709	–
	SD	28	11	8	17	10	7	–	83	27	31	20	16	10	–
	VC	3	1	1	2	1	1	–	7	3	3	2	2	1	–
5	*M*	842	744	707	670	619	–	–	886	764	729	706	657	–	–
	SD	27	18	26	14	8	–	–	21	15	12	7	15	–	–
	VC	3	2	4	2	1	–	–	2	2	2	1	2	–	–
Mean in column	*M*	1 019	770	727	681	624	590	540	1 087	843	801	757	692	662	594
	SD	46	36	22	20	13	9	27	59	30	29	26	20	16	17
	VC	4 8	4 6	3 2	2 8	1 8	1 7	4 5	5 2	3 6	3 6	3 2	2 8	2 3	3 0

exercise loads as during the initial exercise test Resting heart rates were lower in these 5 patients during SR than during AF (Fig 2) Verapamil caused a decrease in resting heart rate in all 5 patients The exercise induced increase in heart rate however was almost identical irrespective of whether the patient had received verapamil or not

Fig 2 shows heart rates at rest and at the different exercise levels in the 5 patients who could be investigated during SR as well as during AF As shown there is a close correlation between heart rates in SR and verapamil treated AF in these patients

Effects of isoproterenol and atropine after verapamil during SR (Table V) Two patients developed sinus bradycardia with nodal escape

Table V *Effect of isoproterenol and atropine upon ventricular rate and regularity during SR and verapamil treatment*

Pat no		RR interval (msec) Before isoproterenol	With isoproterenol	Before atropine	With atropine
1	*M*	950	683	1 031	1 028
	SD	39	15	61	62
	VC	4	2	6	6
2	*M*	1 075	859	1 105	993
	SD	77	64	59	12
	VC	7	7	5	1
3	*M*	1 425	1 225	1 389	1 135
	SD	67	68	72	80
	VC	5	6	5	7
Mean in column	*M*	1 150	922	1 175	1 052
	SD	61	49	64	51
	VC	5 3	5 0	5 3	4 7

rhythm at the time when isoproterenol and atropine tests were to be performed in SR and under the influence of verapamil It is of interest to note that the rate of this escape rhythm differed by only 2–4 beats/min from the rate of the verapamil induced regular ventricular rhythm when the patients were in AF

Side effects

One patient (no 1) developed a moderate but tolerable flushing of his face lasting several hours, after 240 mg of verapamil Although a drop in BP of 10–60 mmHg systolic and 0–15 mmHg diastolic was found almost consistently systolic BP never fell below 95 mmHg and no symptoms or signs attributable to low BP were observed Most patients noted —several with great relief—that their hearts beat regularly under the influence of verapamil

DISCUSSION

The present report confirms our earlier findings that verapamil by mouth results in regular ventricular rhythm even though AF persists (5) Summing up the findings from this and the above study 17 out of 27 patients have demonstrated regular ventricular rhythm after 240 mg of verapamil by mouth All 27 patients have received digitalis in addition to verapamil In fact among a number of reports (1 2 [illegible] 6 7) only two out of altogether 111 patients who [illegible] ventricular regularity after i v verapamil are specifically stated to have had no digitalis treatment (cases 13 and 14 in ref 6)

Both digitalis and verapamil decrease the conduction properties of the AV node although in all probability by distinctly different mechanisms It is possible therefore that digitalis may not be necessary but that it favours the development of a regular ventricular rate after verapamil in patients with AF

The electrophysiological explanation for the verapamil induced regular ventricular rhythm has been unclear (6) The present study illustrates how the regular ventricular rhythm can be maintained although the ventricular rate is changed Although the average effect of exercise atropine and isoproterenol is a gradual loss of regularity some patients maintain the regularity but with increased heart rate Thus our patient 1 (and to some extent even patient 9) maintained a high degree of regularity throughout the exercise test and increased his heart rate from 79 to 99 beats/min Similarly patient 7 increased his heart rate but maintained the pronounced regularity after isoproterenol Patients 6 and 7 reacted in the same way after atropine Thus the ventricular rate seems to be influenced by two factors 1) the inherent properties of the structures of the AV node responsible for the regular ventricular rhythm and 2) a break through of the ordinary AV nodal conduction type during AF These two factors act in a similar manner on physiological and pharmacological stimuli For example sympathetic stimulation and vagal inhibition both lead to an increase in heart rate

Both mechanisms proposed to explain the verapamil induced ventricular regularity (6) (nodal escape rhythm and synchronization of AV nodal conduction) may be relevant to our finding that ventricular rate may change in spite of maintained regularity However another observation strongly supports the hypothesis that the mechanism is total AV block with nodal escape rhythm Thus 2 out of 5 patients who could be re studied during SR developed nodal escape rhythm with almost exactly the same rate as during regular ventricular rhythm in AF and under the influence of verapamil

It should be pointed out that the nodal escape rhythm seems remarkably stable Thus none of our patients or of the patients reported in other studies (1 2 4 6 7) has developed Stokes Adams attacks

The nodal escape rhythm has another property which implies a possible clinical benefit of treatment with high oral doses of verapamil Thus exercise tolerance was not restricted in any patient and the exercise induced changes in heart rate during this rhythm seem to be of the same magnitude as during AF without verapamil and during subsequent SR The isoproterenol and atropine induced changes in heart rate are also of the same magnitude regardless of whether the patient has SR or AF with verapamil

The side effects were few and tolerable This is in contrast to our earlier observations (5) that 6 out of 10 patients had side effects albeit mild and tolerable after a single dose of 240 mg of verapamil by mouth Chronic oral treatment with several daily doses of the same order as used in this study may however well precipitate additional symptoms (5)

There are at least two possible benefits of chronic oral verapamil treatment in doses high enough to induce AV block with nodal escape rhythm The subjective feeling of wellbeing when the heart beats regularly was quite clear in the present study A

haemodynamic improvement is another possible benefit (3) Neither of these benefits is demonstrated in the present report but investigation of these factors necessitates chronic oral as well as acute and chronic haemodynamic studies We believe that the present observations are encouraging enough to warrant such studies

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REFERENCES

1 Cullhed I & Karlsson L Verapamil vid förmaksflimmer Arytmisymposium—Isoptin Göteborg 1972
2 Filias N Verapamil Behandlung bei Herzrhythmusstorungen Schweiz Rundschau Med (Praxis) 63 66 1974
3 Gibson D G Broder G & Sowton E Effect of varying pulse interval in atrial fibrillation on left ventricular function in man Br Heart J 33 388 1971
4 Heng M K Singh B N Roche A H G Norris R M & Mercer J Effects of intravenous verapamil in cardiac arrhythmias and on the electrocardiogram Am Heart J 4 487 1975
5 Khalsa A Olsson S B & Henriksson B Å Effect of oral verapamil on ventricular irregularity in cases of long-standing atrial fibrillation Acta Med Scand 205 39 1979
6 Schamroth L Immediate effects of intravenous verapamil on atrial fibrillation Cardiovasc Res 4 419 1971
7 Schamroth L Krikler D M & Garrett C Immediate effects of intravenous verapamil in cardiac arrhythmias Br Med J 11 660 1972

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The Erythrocyte Sedimentation Rate and Stress

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ABSTRACT The effect of a 75-hour vigil on the erythrocyte sedimentation rate (ESR), i a , was studied in two experiments with 63 healthy male volunteers The ESR was increased at the end of the vigil compared with pre-exposure values The increases did not correlate significantly with concomitant changes in serum triglycerides, free fatty acids, cholesterol or gammaglobulins, except for a significant, negative correlation with cholesterol changes in one of the two studies Although the mechanism for the increases in ESR in response to stressor exposure remains unclear, it is concluded that when using the ESR in clinical practice, allowance should be made for situational factors such as the patient having experienced some stressful days and sleepless nights

Key words stress psychological erythrocyte sedimentation rate cholesterol triglycerides gammaglobulins cortisol

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The erythrocyte sedimentation rate (ESR) is one of the most common measures for screening in clinical medicine A rise in ESR is usually taken to signify some ongoing pathological process in the body but does not convey any specific information about the nature of the process It is generally assumed that the ESR reaction may involve several rather diverse mechanisms e g. decreased hemoglobin (Hb) concentration increased serum concentrations of various elongated proteins (e g fibrinogen gamma and alpha 2 globulins) It has also been suggested that hyperlipidemia with elevated levels of serum cholesterol and triglyceride (TG) might be associated with an increase in ESR A decreased ESR is likewise associated with a variety of situations including an increased Hb concentration and a response to certain anti inflammatory agents such as corticosteroids (2 7 11)

The occurrence of ESR changes in such diverse conditions raises the question whether the ESR also would react as part of the stress response in the Selye sense (8) Stress is generally accompanied by sympathotonic reactions and by an increase in lipolysis and thereby conceivably in ESR If this is so ESR rises should occur in response not only to physical stressors such as infections and inflammation but also to stimuli of a more psychological character (12) If this hypothesis could be confirmed it would imply that a rise or an increased level of ESR in a patient could be associated not only with well known conventional causes but also various environmental influences including psychosocial factors

We therefore investigated whether a controlled stressor exposure—a 75 hour vigil—influenced the ESR and the concentrations of various serum proteins and lipids and whether there is a relation between the ESR and these concentrations A preliminary account of the findings has been published previously (5)

SUBJECTS AND PROCEDURES

The results in this presentation are based on two studies (I and II) performed in nearly the same manner but on different groups of subjects

The subjects of experiment I were 31 army officers and corporals aged 20–44 years (mean 29) They all volunteered (informed consent) and were in excellent physical and mental health as judged from routine medical anamnesis and clinical examination The experiment comprising a stressful vigil started on a Tuesday morning and ended on the Friday afternoon 75 hours later The exposure to stressor included performance on a specially designed shooting range "firing" electronic rifles at small irregularly moving targets (tanks) fitted with photo diodes An authentic battle noise from a tape recorder was amplified to a level of 95 dB-C An unabated 24 hour

Abbreviations ESR=erythrocyte sedimentation rate Hb=hemoglobin FFA=free fatty acids TG=triglycerides

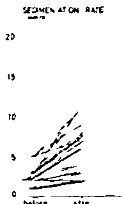


Fig 1 Study I ESR rose from 2 8±0 4 to 7 5±0 9 mm (mean±S E M)

period of such military activity and exposure was followed by a concentrated 15 min period for completion of questionnaires ingestion of a standard meal voiding urine for analysis and attendance to other toilet functions In this manner the experiment was continued for three days without any rest or sleep No other activity but the experimental was allowed The subjects were required to sit on a chair all the time except when voiding or when blood samples were drawn No stimulants or smoking were allowed

Blood samples were taken between noon and 3 p m on either Wednesday or Thursday the week before the experiment (before samples) and on Friday the last day of the vigil ("after samples) In each subject the blood sample was drawn at the same time of day ±30 min preceded by the same food and fluid at the same intervals Further ..ils of the experimental design are given elsewhere (8)

..study II the three hour sessions for 32 officers (mean ..years range 42–64) alternated between the shooting ..and military staff work for a total period of 75 hours The staff work consisted of psychomotor and intellectual tasks of a nature that is also found in many civilian occupations Blood samples were drawn on the day before the vigil started (before) 30 hours after the start (" during") and immediately after the end (after) In other respects this study was the same as study I

METHODS

ESR was determined by the Westergren method (using glass ware and performed by experienced personnel) free fatty acids (FFA) according to Trout et al (15) serum TG according to Kessler and Lederer (6) and serum cholesterol according to Sperry and Webb (13) Blood Hb and serum total protein concentrations as well as the electrophoretic pattern of serum proteins were assessed by routine procedures

The statistical significance of differences was analysed with Student s two tailed *t* test Correlations between variables were measured by linear regression analyses as follows In study I the change between the "before and "after values and in study II the change both between the before and during values and between the "before" and "after" values

The determinations of cholesterol TG and FFA were arranged by L A Carlson King Gustaf V Research Institute Stockholm the determination of fibrinogen by M Blomback Coagulation Laboratory Karolinska Hospital Stockholm and the determinations of serum electrophoresis ESR and Hb concentrations by S A Johansson Department of Medicine Karolinska Hospital Stockholm

RESULTS

ESR

The ESR rose significantly in both studies ($p<0\ 001$ for both) (Figs I and 2) The mean elevation was 4 7 mm in study I and 2 3 mm in study II between before and during with no further change between during and after

Lipids (Table I)

In study I serum TG and cholesterol levels decreased significantly ($p<0\ 01$ and $p<0\ 001$ respectively) No statistically significant correlations with the changes in ESR were found FFA were not determined in this study In study II serum cholesterol levels increased from before to during ($p<0\ 001$) and decreased from during to after ($p<0\ 001$) Serum TG levels fell throughout

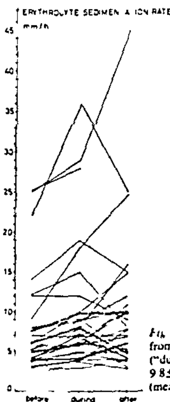


Fig 2 Study II ESR rose from 7 5±1 1 to 9 8±1 4 mm ("during) and remained at 9 8±1 5 mm ("after") (mean±S E M)

Table I *Lipids Hb and proteins determined in studies I and II (mean±S E M)*

	Study I		Study II		
	Before	After	Before	During	After
Cholesterol (mg/100 ml)	193±8	174±7	252±9	289±10	269±11
TG (μmol/ml)	1 00±0 09	0 68+0 05	1.28±0 09	1 06±0 07	0 87±0 06
FFA (mEq/l)			0 36+0 02	0 49±0 03	0 54±0 04
Hb (g/100 ml)	15.5+0 2	14 9±0 2	14 3±0 1	–	14 8±0 1
Total protein (g/100 ml)	8.2±0 06	8 4±0 06			
Albumin (g/100 ml)	5 5±0 04	5 6±0 04			
α 1-globulin (g/100 ml)	0 3±0 01	0 3±0 01			
α-2-globulin (g/100 ml)	0.5±0 01	0 5+0 02			
β-globulin (g/100 ml)	0 6±0 01	0 6+0 02			
γ-globulin (g/100 ml)	1 2+0 03	1 3±0 04			
Fibrinogen (g/100 ml)			0 30+0 01	0 31+0 01	0.31±0 01

the experiment ($p<0\ 001$ for the difference between "before" and "after) Serum FFA levels rose over time ($p<0\ 001$ for the difference between "before and "after') The changes in cholesterol values in study II between "before and after correlated significantly ($r=-0\ 51$ $p<0\ 01$) with the corresponding change in ESR

Hemoglobin (Table I)

Hb concentration fell in study I and rose in study II between before" and after measurements Both of these changes were statistically significant ($p<0\ 001$) but the degree of change was small 0 5 g/100 ml No statistically significant correlations were found in any of the studies between changes in ESR and Hb concentration

Proteins (Table I)

Serum protein electrophoresis was carried out in study I It disclosed that the concentration of total protein rose ($p<0\ 05$) as did gammaglobulins ($p<0\ 01$) *Neither* α_1 α_2 *and* β *proteins nor albu*min and fibrinogen (the latter in study II) showed any change No significant correlations were found between changes in the various proteins and the ESR

DISCUSSION

In both studies exposure to the stressful vigil was accompanied by highly significant albeit moderate increases in ESR Since changes in hematocrit serum levels of gammaglobulins and lipids as well as adrenal activity could be mechanisms in this response the discussion will focus on these possibilities

In study I the serum levels of gammaglobulins showed a small selective increase This increase could not be secondary to the hemoconcentration since the Hb concentration decreased No explanation can be given for this rise of gammaglobulins In contrast to study I the Hb concentration increased in study II possibly indicating some hemoconcentration In spite of this the ESR also rose in study II making it unlikely that changes in hemoconcentration can account for the changes in ESR Furthermore no statistically significant correlations were found between changes in ESR Hb concentrations and gammaglobulin concentrations which suggests that any possible changes in the latter two variables were of minor if any importance as mechanisms in the stressor associated ESR rise

In 1930 Theorell (14) suggested that increases in cholesterol might depress the ESR Such a relationship was later denied by Ohlson and Rundqvist (9) More recently Bottiger (2) found a positive correlation between raised serum cholesterol and TG levels on the one hand and ESR raises on the other However further investigations made it more probable that the increases in both ESR and lipids had a common denominator e g. asymptomatic vascular disease (3) Applying this reasoning to the present results we found that in study I cholesterol levels fell from before" to after whereas ESR rose with no significant correlation between the changes In study II (conducted on older subjects) serum cholesterol increased from "before" to during and then fell from "during to after Here there was a significant correlation to the change in ESR but it had a negative sign

In an in vitro study
were found to decrease the ESR
our study II ESR rose in spite

rise of FFA with no significant correlation between the two variables Briefly we have no basis for any conclusions concerning lipid metabolism or plasma lipids as mechanisms behind the ESR changes

So far we have assumed that it was the stressful vigil which provoked an increase in ESR An alternative could be that the pre-exposure apprehension acted as a more pronounced stressor than the subsequent vigil If this were so it could be argued that pre exposure apprehension actually lowered ESR A subsequent decrease in apprehension during the exposure proper would then be accompanied by a return of ESR to initial levels Hypothetically such a reaction could have been mediated through a corresponding change in endogenous cortisol release Unfortunately no cortisol data are available from studies I and II However in a study carried out exactly as study I but on young and middle aged female subjects (10) we did indeed find that serum cortisol levels prior to and at the beginning of exposure were elevated in relation to the levels during and after the exposure Hypothetically if a similar reaction occurred in studies I and II it would have coincided with a corresponding rise in ESR It is well known that administration of glucocorticoids reduces the elevated ESR in many clinical conditions (1)

In conclusion a three-day stressful vigil was accompanied by a highly significant albeit moderate increase in the ESR but nothing definite can be said about the mechanisms behind these reactions Although the correlations between ESR changes and other variables measured do not allow any conclusions about the role of the latter they may still have played a role e g if there is a time lag between the various sets of reactions

Whatever the mechanisms the fact that increases in ESR up to 20 mm have been shown to accompany exposure to a stressful vigil should be drawn to the attention of clinicians It implies that relatively short lasting environmental exposures can provoke increases in ESR without the latter necessarily being an index of a pathological process as is usually assumed In this sense ESR reactions resemble others such as serum iron protein bound iodine serum cholesterol TG and FFA ECG and others (8) It follows that environmental factors such as those in our studies (e g leading to sleep deprivation apprehension fatigue etc) should be taken into account when interpreting laboratory data in clinical practice

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REFERENCES

1 Albrecht H J Zur unterschiedlichen Hemmbarkeit der Blutkörperchensenkung in vitro durch Prednisolon bei verschiedenen Krankheiten Blut 21 371 1970
2 Bottiger L E Erythrocyte sedimentation rate and plasma lipids Acta Med Scand 193 53 1973
3 Bottiger L E Carlson L A Ekelund L G & Olsson A G Raised erythrocyte sedimentation rate in asymptomatic hyperlipidaemia Br Med J 2 681 1973
4 *Ehrly A M Gramlich F & Muller H E Uber den* Einfluss von Lipiden insbesondere von unveresterten Fettsauren auf den Mechanismus der Blutkörperchensenkungsgeschwindigkeit Klin Wochenschr 49 943 1965
5 Froberg J Karlsson C-G Levi L & Lidberg L Physiological and biochemical stress reactions induced by psychosomatic stimuli In Society stress and disease vol 1 The psychosocial environment and psychosomatic disease (ed L. Levi) pp 280-295 Oxford University Press London New York and Toronto 1971
6 Kessler G & Lederer H Fluorimetric measurement of triglycerides In Proceedings from the Technicon Symposium Automation in Analytical Chemistry pp 341 344 1965
7 Lascari A D The erythrocyte sedimentation rate Pediatr Clin North Am 19 1113 1972
8 Levi L Stress and distress in response to psychosocial stimuli Acta Med Scand (Suppl) 528 1972
9 Ohlson B & Rundqvist O Über die Bedeutung der Plasmalipoide für die Suspensionstabilität des Blutes Biochem Z 247 249 1932
10 Palmblad J Cantell K Strander H Froberg J Karlsson C-G Levi L Granstrom M & Unger P Stressor exposure and immunological response in man Interferon producing capacity and phagocytosis J Psychosom Res 20 193 1976
11 Ruhenstroth Bauer G Mechanismus und Bedeutung der beschleunigten Erythrocytensenkung Klin Wochenschr 44 533 1966
12 Schneider R A The relation of stress to clotting time relative viscosity and certain other biophysical alterations of blood in the normotensive and hypertensive subject Proc A Res Nerv Ment Dis 29 818 1950
13 Sperry W M & Webb M A revision of the Schoenheimer Sperry method for cholesterol determination J Biol Chem 187 97 1950
14 Theorell H Studien uber die Plasmalipoide des Blutes Biochem Z 223 1 1930
15 Trout D L Estes L H Jr & Friedberg S J Titration of free fatty acids of plasma a study of current methods and a new modification J Lipid Res 1 199 1960

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Follow-up Studies of Joint Complications in Yersiniosis

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ABSTRACT In 1971–73, 60 patients were hospitalized for an infectious disease caused by Y enterocolitica serotypes III and IX or Y pseudotuberculosis serotype I In the spring of 1977 a follow up study was carried out with 52 of these patients one patient having died from intestinal perforation evidently resulting from yersiniosis-induced amyloidosis and colitis The follow up study showed that two patients had symptoms of sacroiliitis one had developed an apparent rheumatoid arthritis after yersiniosis and one had joint pains and a high serum rheumatoid factor titer with no objective joint changes All results from kidney and liver studies were normal No brucellosis antibodies were found in the follow up study Twelve patients had antibody titers against Y enterocolitica serotype III

Key words infection yersinia reactive arthritis
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The gram negative rods Yersinia enterocolitica and Yersinia pseudotuberculosa usually produce fever diarrhea and abdominal cramps in clinical infections Some patients develop erythema nodosum arthritis, carditis terminal ileitis mesenteric lymphadenitis glomerulonephritis hepatitis etc (1 2 3 4 5 8 11) Those who have contracted Yersinia infection and have the HL A27 gene run 50 times the risk of developing postinfection reactive arthritis compared with those who lack this gene (4) At the onset of the disease the etiologic agent can be separated e g from the feces or blood but the diagnosis is generally based on finding specific antibodies in the blood (11) Yersiniosis is evidently rather common because e g in Denmark and Germany titers of Yersinia enterocolitica antibodies exceeding 1 80 were found in 5-6% of routine clinical immunologic laboratory specimens (6 9) It is evident that at least in Europe yersiniosis is at present the most important infection producing reactive arthritis and thus has superceded rheumatic fever complications of streptococcal infections these being two diseases which are symptomatically very similar to each other (7 11)

Information on the prognostic significance of human yersiniosis is very limited as is the case with many other reactive arthritides In this paper we describe the results of a follow up study of our patients from 1971–73 The primary results have been reported earlier (11)

PATIENTS AND METHODS

Our primary study comprised 60 patients all considered to have yersiniosis with positive Y enterocolitica or pseudotuberculosa antibody titers of at least 1 160 Of the original patients 52 were brought to the follow up studies at the Department of Internal Medicine Turku University Central Hospital during the spring of 1977 (Table I) One patient had died during the follow-up period but detailed information was available on the course of his disease The average follow up period was 5 years (range 3½–7)

The control group consists of 53 patients matched for age and sex They were treated in the same department because of urinary distresses Their urography films were used for the lumbal spine and sacroiliac joint evaluation If necessary special projections were taken Chest X ray examination ECG routine laboratory tests and clinical examination were performed in all patients Special attention was paid to the medical history of the last five years in these patients

In the follow-up study the standard clinical examination was amplified with a physical examination of the joints X rays of all joints where the patients complained of symptoms and of the sacroiliac and hip joints of all the patients except one pregnant woman In the same way chest films and ECGs were taken of all patients Hb level Hct total leukocyte count ESR (Westegren) urine sediment serum creatinine antibody titers against Y enterocolitica serotypes III and IX and Y pseudotuberculosa serotype I antistreptolysin 0 titer and the Bang test for brucellosis were recorded in all patier [illegible] tion the Waaler Rose test and Latex fixation [illegible] in all patients in order to der[illegible] ie rheumat

Table I Data on the patients in follow up study

Pat no	Sex	Age (y)	Follow up time (y)	Yersinia antibody titers Primary phase	Follow up	Remarks
				Y entero colitica III	Y entero-colitica III	
1	♀	44	5 8	640	640	Rheumatoid arthritis
2	♀	24	3 8	1 280	640	nodal beats in ECG
3	♀	34	6 5	1 280	640	
4	♂	58	5 7	20 000	320	
5	♀	34	4 9	160	320	
6	♂	53	5 0	5 120	320	
7	♀	49	4 1	320	160	
8	♂	44	5 0	5 120	160	Bilateral hilar adenopathy
9	♀	59	4 9	2 560	160	
10	♀	26	4 8	640	80	Mild mitral regurgitation
11	♀	58	5 0	640	80	
12	♀	36	3 7	160	80	
13	♀	62	4 2	10 240	–	
14	♀	62	5 7	10 000	–	
15	♀	69	3 6	5 120	–	
16	♂	44	4 0	5 120	–	
17	♀	44	7 0	5 120	–	Sacroiliitis
18	♀	25	6 7	2 560	–	
19	♂	75	6 0	2 560	–	
20	♂	31	6 0	2 560	–	Sacroiliitis
21	♂	36	4 0	2 560	–	
22	♂	24	5 6	2 560	–	
23	♀	42	5 9	2 560	–	
24	♂	45	6 3	2 560	–	
25	♀	54	5 3	2 560	–	
26	♂	25	3 7	1 280	–	
27	♀	45	5 4	1 280	–	Sacroiliitis
28	♀	29	6 2	1 280	–	
29	♂	49	5 0	1 280	–	
[illegible]	♀	47	5 8	1 280	–	
[illegible]	♂	34	6 2	1 280	–	
[illegible]	♀	22	3 7	640	–	
[illegible]	♀	62	4 7	640	–	
4	♀	22	6 6	640	–	Waaler Rose 1024 arthralgia
35	[illegible]	42	5 9	640	–	
36	♀	32	5 8	640	–	
37	♂	28	3 8	640	–	
38	[illegible]	55	4 3	640	–	
39	♂	37	5 0	640	–	
40	♀	25	4 0	320	–	
41	♀	29	5 7	320	–	
42	♀	22	4 6	160	–	
43	♀	79	5 7	160	–	
				Y enterocolitica IX		
44	♂	48	3 7	2 560	–	
45	♂	53	5 1	1 280	–	
46	♂	44	3 5	1 280	–	
47	♀	39	4 2	1 280	–	Rheumatoid arthritis
48	♀	47	4 2	160	–	
49	♂	21	5 0	160	–	
50	♀	74	4 7	160	–	
				Y pseudotuberculosa I		
51	♀	47	3 8	2 560	–	
52	♂	31	4 1	1 280	–	Synovectomia genu
53	♂		4 0	320		Exitus

Table II *Duration of arthritis in the primary phase in 27 patients participating in the follow up*

Duration (mo)	No of pats
1	4
1–3	9
3–6	8
6–12	4
>12	2

factor Serum alkaline phosphatase (ALP) and aspartate aminotransferase (ASAT) were also studied in all patients During the clinical examination special attention was paid not only to the spine and joints but also to auscultation of the heart with a view to comparisons with the findings from the acute period

Patient 53 was 28 years old when contracting yersiniosis as a feverish diarrhea and he developed erythema nodosum arthritis and negative T waves in the ECG The disease rapidly developed into a severe chronic colitis requiring colectomy two years later During 1974 the nephrotic syndrome developed evidently on the basis of amyloidosis The patient died in Dec 1975 from small intestinal perforation caused by amyloidosis The cause of the disease during the primary phase was Y pseudotuberculosis I

RESULTS

Joints and the back

In the primary phase 33 patients out of 60 had arthritis 27 of these participated in the follow up study The duration of arthritis in the primary phase according to the history obtained in the follow up study appears in Table II

One patient (no 52) who underwent synovectomy 11 months after the initial infection because of chronic hydrops had no joint symptoms at the time of the follow up study He had negative rheumatoid serology disappearance of Yersinia antibodies from the blood and an ESR of 17 mm/h

In the follow up study three patients had subjective symptoms consistent with the primary phase of ankylosing spondylitis In one of them (no 20) the diagnosis can be considered rather certain He was 31 years old at the time of the follow up had suffered from arthritis in the primary phase of yersiniosis and in 1975 he suffered from scleritis of the left eye At the follow up study the hip joint was painful and stiff the sacroiliac joints were tender to percussion radiologically a distinct unilateral sacroiliitis was seen the ESR was 29 mm/h Yersinia antibodies were negative rheumatoid factor could not be demonstrated and the serum ALP was slightly elevated The second patient (no 17) also had arthritis during the primary phase and now complained of arthralgic symptoms The movements of the back were normal one sacroiliac joint showed radiologic evidence of sacroilitis ESR was 11 mm/h the other joints were radiologically normal and Yersinia antibodies and rheumatoid factor tests were negative The third patient (no 27) had also had arthritis during the primary phase In the follow up study she complained in particular of back pain which predated her yersiniosis but had impaired Her back was stiff and painful with percussion tenderness at the sacroiliac joints, although the radiologic findings were more consistent with degenerative than inflammatory disease The ESR and serologic tests were normal Radiological signs suggesting sacroilitis were not seen in the control group

Development toward rheumatoid arthritis occurred in three female patients The first patient (no 1) had arthritis during the primary phase which has not disappeared but remains symptomatic She was found to have swelling and soreness in the metatarsophalangeal joints of both feet and in the right wrist Soft tissue swelling but no erosions were seen radiologically The serum rheumatoid factor could not be confirmed instead the Y enterocolitica III antibody titer was still the same as in the primary phase 1 640 ESR was 9 mm/h The patient has been treated for a year as a rheumatoid arthritis case The second patient (no 47) had pain in the metacarpophalangeal joints where slight erosions were seen radiologically She had arthritis during the primary illness The Waaler Rose and Latex fixation tests were both positive ESR was 38 mm/h Yersinia antibody tests were negative During the primary phase of the disease she had had biphasic T waves in the unipolar precordial leads but the ECG had become completely normal In this case the diagnosis of rheumatoid arthritis can be considered certain but the relation of the situation to yersiniosis is complicated by the fact that the patient had complained of joint symptoms which were not studied before her yersiniosis Thus one cannot exclude the possibility that the disease predated her yersiniosis The third patient (no 34) was at follow up a 22 year-old pregnant woman who in the primary phase of yersiniosis had polyarthritis and a

Waaler Rose titer of 64 (still considered negative) Arthritis was treated for 4 months but since then the patient has continued to experience migratory arthralgic symptoms In the follow up study no objective joint changes were noticed but the Waaler Rose titer had increased to 1 024 ESR was 8 mm/h No radiologic studies were performed because of her pregnancy (3rd month) There were no cases in the control group whose rheumatoid arthritis or sacroiliitis had developed during the last five years

The primary material included two patients with previously diagnosed rheumatoid arthritis and one patient with systemic lupus erythematosus (SLE) predating the onset of yersiniosis The development of these diseases after yersiniosis did not differ from the expected course In the control group there was one patient with rheumatoid arthritis and two with SLE The diseases had started before the last five years In the follow up study 7 patients complained of joint and back symptoms which they had had before yersiniosis These lesions were of degenerative origin and had progressed radiologically at follow up The same number (seven) of degenerative changes was seen in the control group

Heart

In the primary phase 4 patients had had ECG changes possibly caused by carditis The patient described above who died from colitis complications during the surveillance period was also included in this group

In the follow up study one patient (no 2) was found to have partial RBBB as well as temporary ventricular ectopic beats the situation being the same as in the primary phase The other two patients with carditis in the primary phase were now electrocardiographically normal One 26-year-old female patient (no 10) who had elevated Y enterocolitica III antibody titer (1 640) and antistreptolysin 0 titer (1 1400) in the primary phase had a long lasting rolling murmur of mild mitral regurgitation at the apex and ST segment depression in leads II and III at the follow up The chest film was completely normal and the patient was subjectively asymptomatic Two patients in the control group had apical systolic murmur suggesting mitral regurgitation ectopic beats and RBBB were recorded once

Other studies

Excluding SLE patients all the urinary sediment findings and the serum creatinine values were normal The patient with glomerulonephritis on renal biopsy in the primary phase was now completely asymptomatic

All the patients had serum ASAT and ALP within normal ranges (with the exception of the patient mentioned above with evident ankylosing spondylitis and slightly elevated ALP)

Table I lists the 12 patients who were found to have Yersinia antibody titers against Y enterocolitica serotype III in the follow up study

Serotype IX of Y enterocolitica cross rea ts with Brucella No brucellosis antibodies were found in the follow up Antistreptolysin 0 titer was elevated in the primary phase in 6 patients but only in one in the follow up study

A male patient (no 8) in the Yersinia group had bilateral hilar adenopathy with negative Kveim test and positive tuberculin test

DISCUSSION

The results of our follow up study showed a more favorable outcome for yersiniosis than expected However in four patients out of 53 there was an association between yersiniosis and the subsequent development of sacroiliitis and rheumatoid arthritis. Symptoms suggesting rheumatoid arthritis and sacroiliitis were present in one patient each during the follow up They had already suffered from symptoms of same kind before the onset of yersiniosis but after this the symptoms became worse In the control group no radiological signs were found suggesting rheumatoid arthritis or sacroiliitis The number is considerably smaller than in another Finnish series (4) where 64 patients were studied for an average of 3 years 9 months after yersiniosis with findings of bilateral sacroiliitis in 7 unilateral sacroiliitis in 7 and pain on joint motion in 21 cases The difference is possibly due to the selection of patients in the above mentioned study nearly all of whom developed reactive arthritis in connection with acute yersiniosis Our study describes the fate of an unselected yersiniosis series The seven patients who failed to take part in our follow up study all had arthritis in the primary phase Larsen et al (10) found Y enterocolitica serotype III antibodies with titers of ≥ 1 10 in 42% of 355 patients suffering from rheumatoid joint disease and SLE In their patient group titers of ≥ 1 80 occurred in 20% and the authors stated some acute cases of rea

tive Yersinia arthritis are not resolved but recur and develop gradually into chronic joint damage i e that the most frequent cause of acute arthritis is also the most frequent initiator of chronic collagenosis It is relevant that very low titers were involved in their patients titers which generally occur in healthy blood donors (1)

Reiter s syndrome can also be considered a form of reactive arthritis It was found in a study of 100 *Reiter patients 20 years after the acute syndrome* (12) that the most frequent late changes were rheumatoid spondylitis (32%) chronic arthritis (18%) and iritis (7%) only 20% of the cases being entirely asymptomatic Reiter s syndrome is as strongly associated with the HL A27 gene as reactive arthritis (2) following yersiniosis Thus it is not unexpected that the prognosis is similar for reactive arthritis following yersiniosis and Reiter s syndrome respectively In this connection it is interesting to mention a theory according to which the HL A27 cell surface antigen would cross react with the Yersinia antigene which might be an antigen common to the enterobacteria an enteric common antigen (12)

A five year follow up is not long enough to determine the frequency of cardiac valvular defects after yersiniosis In our study one case of unconfirmed mitral regurgitation was found in the follow up the patient having had a very high antistreptolysin 0 titer in the primary phase which is difficult to explain It must also be pointed out that apical murmur was heard in two patients in the control group In the follow up study the few rhythm disturbances were of non specific etiology

We consider it unexpected that as many as 12 patients still had antibody titer to Y enterocolitica serotype III but no elevated titer was found against Y pseudotuberculosa The persistence of antibodies for two years has been reported earlier (1) In this follow up study it is noteworthy that only one patient with joint symptoms and one suffering from rhythm disturbances belonged to this group Our patient who developed bilateral hilar adenopathy after yersiniosis is analogous to the cases reported by Sairanen (13)

We consider our results show that the 5 year prognosis for unselected human yersiniosis cases is quite favorable but that there is a tendency to develop rheumatoid arthritis and ankylosing spondylitis This tendency appears to be much more pronounced when reactive arthritis is associated with yersiniosis in the primary phase (4) The frequency of development of cardiac valvular defects after yersiniosis is not known

REFERENCES

1 Aho K Ahvonen P Lassus A Sievers K & Tulikainen A Yersinia arthritis and related disease clinical and immunogenetic implications Arthritis Rheum 17 341 1974

2 — HL A27 in reactive arthritis A study of yersinia arthritis and Reiter s disease Arthritis Rheum 17 521 1974

3 Ahvonen P Human yersiniosis in Finland I Bacteriology and serology Ann Clin Res 4 30 1972

4 — Human yersiniosis in Finland II Clinical features Ann Clin Res 4 39 1972

5 Forsstrom J Viander M Lehtonen A & Ekfors T Yersinia enterocolitica infection complicated by glomerulonephritis Scand J Infect Dis 9 253 1977

6 Knapp W Yersiniosen Med Welt 28 1586 1977

7 Laitinen O Leirisalo M & Allander E Rheumatic fever and yersinia arthritis Criteria and diagnostic problems in a changing disease pattern Scand J Rheumatol 4 145 1975

8 Larsen J H Yersinia enterocolitica infections and arthritis In Infection and immunology in the rheumatic disease (ed D C Dumonde) p 133 Blackwell Scientific Publications Oxford London Edinburgh and Melbourne 1976

9 — Yersinia enterocolitica infektioner og deres hyppigste komplikationer Ugeskr Laeger 139 2627 1977

10 Larsen J H Jarner D & Jarløv N V Yersinia artrit og kronisk kollagenose I Akut yersinia artrit og forekomsten af specifikke yersinia antistoffer ved kronisk kollagenose Ugeskr Laeger 139 1478 1977

11 Leino R & Kalliomaki J L Yersiniosis as an internal disease Ann Intern Med 81 458 1974

12 Makela P H & Mayer H Enterobacterial common antigen Bacteriol Rev 40 591 1976

13 Sairanen E Hilar adenopathy with Yersinia infection Ann Intern Med 80 673 1974

14 Sairanen E Paronen I & Mahonen H Reiter s syndrome A follow-up study Acta Med Scand 185 57 1969

Acta Med Scand 205 527–534 1979

Left Atrial Myxoma Moving from Right Atrium to Left Ventricle

Non Invasive and Invasive Techniques and Surgical Findings

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ABSTRACT A case of left atrial myxoma, prolapsing through a large atrial septal defect during systole and through the mitral valve orifice during diastole, is presented To our knowledge this is the third such case and only the second one in which the diagnosis was made before operation Echocardiography and phonocardiography were of great value in establishing the diagnosis of left atrial myxoma the features before and after operation are presented In this patient the "swinging" of the tumor in the left atrium and in the left ventricle was echocardiographically visible Correlations of tumor movement and heart sounds could be made The diagnosis of a 36% left to-right shunt on atrial level could not be made with the help of non invasive techniques alone, cardiac catheterization revealed the shunt The role of non invasive techniques and of cardiac catheterization is discussed, together with a review of the relevant literature

Key words myxoma echocardiography phonocardiography atrial septal defect

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Primary tumors of the heart are rare most of them being myxomas About 75% of the myxomas are located in the left atrium A myxoma of the heart can be found together with acquired and congenital malformations of the heart (10 17 42) A left atrial myxoma associated with a left to-right shunt on atrial level has been reported before by Cumming and Finkel (13) and Malm et al (26) The former diagnosis was made at autopsy the latter before operation

Phonocardiography and pulse recording can be very useful in establishing the diagnosis of left atrial myxoma (12 16 20 21 23 24 25 27 31 34 35 36 39 40 43 44) Sometimes a diastolic murmur mimicking a mitral stenosis is present The murmur can change in loudness from day to day or with the changing *position* of the patient Other abnormalities such as a systolic murmur a loud first heart sound a late opening snap a loud pulmonary second heart sound and a third heart sound the so called tumor plop can also be heard

The difficulties of the non invasive preoperative diagnosis of a myxoma have been ameliorated since the introduction of echocardiography (31 35 37 40 41 43) a mass of echoes will be found behind the anterior mitral valve leaflet in ventricular diastole Sometimes these echoes are also visible behind the posterior wall of the aorta in the left atrium

The patient s history together with auscultatory and laboratory findings give mostly an indication of the possibility of the diagnosis of left atrial myxoma *dyspnea on exertion symptoms caused by systemic* emboli arrhythmias when the patient is lying in left lateral position progressive weakness chest pain and low grade fever (1 2 7 8 18 28 30 33 38) Laboratory findings include elevated ESR anemia and elevation of the gammaglobulin content of the blood

CASE REPORT

A 60-year old woman was admitted to our hospital after having experienced the following symptoms for about 12

Abbreviations S I = first heart sound S II = second heart sound II P = pulmonic closure sound II A = aortic closure sound

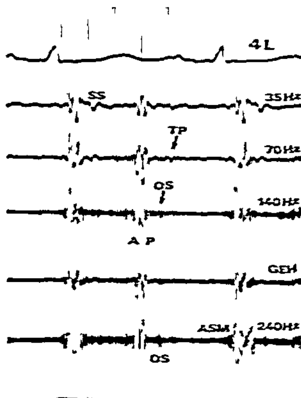


Fig. 1. Phonocardiogram, registration on the 4th rib on the left side of the sternum. A prolonged first and a split second heart sound are recorded. A = aortic component; P = pulmonic component of the second heart sound. An opening snap (OS) is recorded in 140 Hz and 240 Hz. A tumor plop (TP) is present in all frequencies, but clearest in 70 Hz. A systolic sound (SS) is visible early in systole. A holosystolic murmur with a late accentuation is recorded, as well as a soft diastolic murmur (ASM).

months, loss of weight, dyspnea on exertion, progressive weakness, slight ankle edema, intermittent low-grade fever and palpitations. Before this period she had had no complaints. There was no prior history of rheumatic fever, chest pain or syncope.

At physical examination she looked healthy and had a normal skin color. The temperature was 37.3°C, BP 140/80 mmHg and there were no evident signs of heart failure. The left ventricular impulse was normal and no right ventricular impulse was palpable. The first heart sound (S I) was single and loud, the second heart sound (S II) was split normally and the pulmonic component of S II (II P) was accentuated. A blowing, soft systolic and an early diastolic murmur were heard on the left and right side of the lower sternal border.

Chest X-rays showed a moderate enlargement of right and left ventricle and slight pulmonary venous congestion. The pulmonary segment was enlarged, as were the right and left atrium. The ECG and VCG showed sinus rhythm, right axis deviation, right ventricular hypertrophy and right atrial enlargement.

ESR was initially 41 mm/1 h, later 61 and 120 mm/1 h. Hb was 11.9 g/100 ml and Hct 36%. WBC, platelets and plasma proteins were normal.

Because of dyspnea, the patient was observed in the Department of Pulmonary Diseases, where no explanation was found for the complaints or for the abnormal laboratory findings. However, it was noticed that dyspnea and irregular heart rhythm became evident or deteriorated when the patient positioned herself in the left lateral position.

Because of suspicion of a left atrial myxoma, the patient was transferred to the Department of Cardiology, where phonocardiography, echocardiography and cardiac catheterization were performed.

METHODS

Phonocardiographic tracings were photographically recorded in held expiration with a Hellige phonocardiograph (Maass-Weber filters) with a paper speed of 100 mm/sec. Positions of the microphone were on the second and fourth rib on the right side of the sternum and on the second, third, fourth and fifth rib on the left side of the sternum, at the apex, also in left lateral position and on a point between the fourth rib parasternal and the apex, in conjunction with an ECG. Jugular venous tracings and carotid pulse tracings were made in the supine position. An apex cardiogram was recorded in left lateral position.

The echocardiogram was recorded with an Ekoline 20 A echocardiograph, using a 2.25 MHz transducer with a diameter of 12 mm and an repetition rate of 1 000 pulses/sec. Photographic recordings were obtained using a Cambridge strip chart recorder with a paper speed of 75 mm/sec; a sweep was made with a paper speed of 50 mm/sec. Simultaneously and ECG was recorded, as was an external phonocardiogram using a Cambridge amplifier [illegible] 77 and type 5643 microphone. Registrations were made from the aorta with left atrium, from the mitral valve region and from the left ventricular posterior wall with the patient in left lateral position. A sweep from the aortic region to the left ventricular posterior wall was also made.

During cardiac catheterization, five dilution curves were made according to the technique described by Mook (29) and ten Hoor (22).

RESULTS

The phonocardiogram (Fig. 1) showed a loud, prolonged SI and a splitting of SII. II P was too loud. There was a diastolic sound in the high frequencies, 90 ms after the aortic closure sound (II A), and a soft diastolic sound in the low frequencies, 150 ms after II A. An ejection sound of the aorta was recorded. Immediately after this sound, early in systole, a sound was recorded in the low frequencies, louder in the basal than in the apical region. There was a soft holosystolic murmur in the mid and high frequencies, with a late systolic accentu-

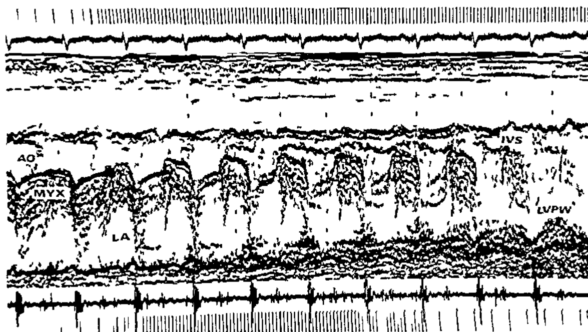


Fig 2 Echocardiogram registration via the 4th intercostal space at the left side of the sternum a sweep from aorta (*AO*) to the left ventricular posterior wall (*LVPW*) is made *LA* left atrium *IVS* interventricular septum *MYX* = myxoma

tuation the loudest on the lower left sternal border A short early diastolic murmur was recorded at the lower right and left sternal border A soft atrio systolic murmur existed at the apex The jugular venous tracing showed a normal pattern except for the *x* descent which showed an *s* wave as was seen in the liver tracing The carotid tracing was normal registration with respiration showed a re

Table I Hemodynamic data obtained at cardiac catheterization

	Pressure (mmHg)	Oxygen saturation (%)	
Superior vena cava		70	
Inferior vena cava		76	
Right atrium	5	85	
Right ventricle		82	
Pulmonary artery	75/20	82	
Pulmonary capillary wedge			
Pressure (mean)	27		
v wave	52		
Calculated left to right shunt (%)			36
Cardiac output (l/min)			4 6
Pulmonary flow (l/min)			7 2

spiration dependent splitting of S II The ejection time index was abnormally short 377 ms The pre ejection period (corrected) was 157 ms The Q(ECG) S I interval was 80 ms The apex cardiogram had a poor definition of the diastolic events There was a small anacrotic notch together with a prolonged S I The systolic retraction of the apex cardiogram was early and sharp with a midsystolic minimum

The echocardiogram (Fig 2)—routinely recorded in all patients undergoing cardiac catheterization—showed a normal septal thickness with a normal direction of movement There was a normal aspect of the aorta Behind the aorta in the slightly enlarged left atrium there was a dense mass of echoes from the posterior wall of the aorta to the left atrial wall during ventricular systole occupying about half of the left atrium during ventricular diastole A sweep from the aortic region to the mitral valve and left ventricular posterior wall showed a diminished E–F slope of the anterior mitral valve leaflet behind this structure the dense mass of echoes could be seen in the mitral valve orifice also till the moment when the left ventricular posterior wall was visible

The deepest part of the echoes in the left atrium during ventricular systole did not coincide with S I

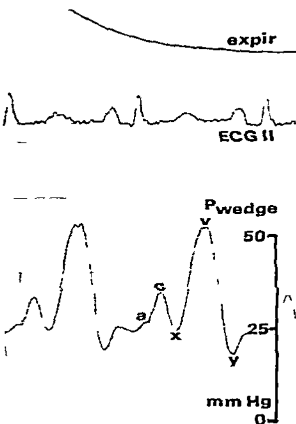


Fig 3 Pressure registration in wedge position (P_{wedge}) with measurements made in the expiratory phase The typical pattern caused by a left atrial myxoma is present a small *a* wave a pronounced *c* wave and a very tall [illegible] with a rapid v-descent.

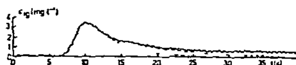


Fig 4 Dye dilution curve obtained by injection of 2.5 mg indocyanine green into the main pulmonary artery at zero time ($t=0$) and continuous sampling from the right femoral artery through a linear reflection densitometer cuvette Besides a normal peak this curve shows an early recirculation peak due to a 36% left to-right shunt.

but with the sound that existed immediately after SI

A third heart sound" was seen after the maximal opening of the mitral valve coinciding with the moment when the visible movement of the tumor in the left atrium stopped rather abruptly

Cardiac catheterization showed the features presented in Table I

The indirect measurement of the left atrial pressure (catheter in wedge position) showed a mean pressure of 27 mmHg a small *a* wave prominent *c* and *v* waves and a rapid v-descent (Fig 3) Dye dilution curves revealed a left to-right shunt of 36% of the pulmonary circulation (Fig 4) which—with respect to the oxymetric data—appeared to be on atrial level

The levophase of pulmonary angiography showed a large filling defect in the left atrium during ventricular systole (Fig 5)

The clinical diagnosis was a left atrial myxoma an atrial septal defect with a left to-right shunt pulmonary hypertension and a moderate incompetence of the tricuspid valve

The patient was operated on seven days after cardiac catheterization Opening of the right atrium revealed a large secundum type atrial septal defect that permitted a left sided gelatinous semi transparent lobular mass to prolapse 2–3 cm into the right atrium (Fig 6) The pedicle of the tumor was situated on the left side at the rim of the fossa ovalis The tumor could move freely up and down through the mitral valve orifice The tumor was removed with the remainder of the interatrial septum

The septal defect was closed with a teflon felt patch After closing the right atrium the pressure curve showed high systolic peaks as can be seen in cases of tricuspid regurgitation The right atrium was re-opened but no traumatic lesion of the tricuspid valve was seen the valve had a normal aspect

The postoperative course was uncomplicated The patient was discharged 13 days after operation in excellent general condition

The tumor measured 6×4×2 5 cm and weighed 41 5 g The microscopic appearance was that of a myxoma.

After the operation phonocardiogram and echocardiogram were repeated

The postoperative phonocardiogram showed normal SI and SII and an ejection sound of the aorta. The IIP was soft and of low frequency There was no splitting of SII A low frequency early diastolic sound and a fourth heart sound were visible in all frequencies (35–240 Hz) followed by a soft atriosystolic murmur A very soft early systolic murmur was registered at the apex

The jugular venous tracing showed a prominent *v* wave The carotid tracing was normal The ejec





Fig 5 The levophase of pulmonary angiography Left the heart n ventricular diastole an enlarged left atrium s f lled with contrast R ght the heart n ventricular systole there s a large f lling defect in the left atrium caused by a myxoma (arrows)



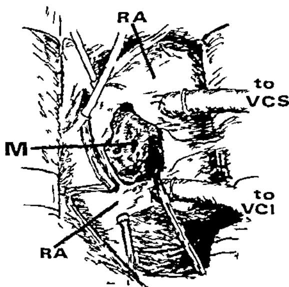


Fig 6 The right atrium during operation The right atrium (*RA*) has been opened and f om th s po nt a large myxoma (*M*) originating from the left atrium s vis ble through a large atrial septal defect *VCS* vena ca a superior *VCI* = vena cava nferior

tion time index was shortened as before 367 ms The Q(ECG) S I interval was now shorter, 60 ms

The apex cardiogram was unchanged there was a poor definition of the diastolic events, an anacrotic notch at the moment of S I and a sharp systolic retraction with a midsystolic minimum

The postoperative echocardiogram showed a normal right ventricle and a paradoxical movement of the interventricular septum The anterior and posterior mitral valve leaflet showed a normal pattern with a normal E–F slope A small echo free space between epicardium and pericardium was seen The left atrium was still slightly enlarged

DISCUSSION

The remarks in the literature on phonocardiographic signs in left atrial myxoma are not uniform This is not very surprising myxomas of different forms and weights in different hearts must give rise to different findings Mostly a loud prolonged S I was found sometimes an opening snap (24) and sometimes a so called tumor plop The apex cardiogram mostly showed a prominent notch in the upstroke (12) corresponding to the loud S I

The S I was loud and prolonged in our patient there was a soft opening snap and also a tumor plop An early systolic sound coinciding with the deepest part of the tumor on the echocardiogram was also ...d giving rise to the suspicion of an early ...lic tumor sound

The splitting of S II might have been caused by the atrial sepatl defect and/or the left atrial myxoma In the case of a great left atrial myxoma, there will be left atrial inflow obstruction of the pulmonary veins which causes splitting of S II (36)

After operation S I became softer the Q(ECG) S I interval became shorter the splitting of S II disappeared as did the opening snap The II P became soft The murmurs disappeared except for a very soft atriosystolic and an early systolic murmur

Echocardiography proved to be of great value a mass of echoes behind the anterior mitral valve leaflet is a characteristic pattern The E–F slope of this leaflet is diminished suggestive of mitral stenosis, but the leaflet excursion is too large and the posterior leaflet is not visible

Many mistakes can be made insufficient rejection or damping or too high gain can give false positives too low gain false negatives A clot can mimic the picture which is why a sweep from the aorta to the left ventricular wall has also to be made

It is not always possible to perform a satisfactory registration due to deformity of the chest or because of pulmonary disease

Our patient showed a very clear echocardiogram the swinging of the left atrial myxoma could be seen from the left atrium to the left ventricle and back

During ventricular diastole, when the tumor has moved downward to the left ventricle only the smaller top of the tumor could be seen behind the aorta as a mass of echoes occupying about half of the left atrium During ventricular systole the tumor was seen in nearly the entire left atrium Directing the transducer downward, the echoes filled up the mitral valve orifice also at the moment when the left ventricular posterior wall became visible, which means that the tumor was in the left ventricular cavity at that moment After operation a normal pattern of the mitral valve was seen

The atrial septal defect could not be diagnosed by non invasive techniques despite the fact that there was a 36% left to right shunt One would expect a respiratory independent splitting of S II there was a splitting (that also could have been caused by the myxoma) but it was respiratory dependent The jugular venous tracing showed a normal aspect of *a* and *v* waves confirmed by the liver tracing In cases of atrial septal defect the *a* wave mostly measures less than 1 5×the *v* wave in this tracing this was more The direction of movement of the interventricular septum was normal before operation backwards during ventricular systole In cases of an atrial septal defect (right ventricular volume overload) a paradoxical movement might be expected The postoperative echocardiogram did show this but tricuspid insufficiency must have been the cause

Cardiac catheterization showed a higher oxygen saturation in the left atrium than in the vena cava The indirect left atrium pressure curve (wedge position) showed a small *a* wave, prominent *c* and *v* waves and a rapid *y* descent as described before (9 36) from direct left atrial pressure measurements by means of transseptal catheterization

The indirect pressure curve gives as much information as the direct one because of the risk of systemic embolization of the tumor a transseptal procedure should be avoided when the presence of a left atrial tumor is suspected (31)

It seems logical to assume that the rapid y descent results from the tumor mass itself when it leaves the left atrium the prominent c wave must have been caused by a sudden increase in the volume of the left atrium when the tumor moves backward from the left ventricle the tall v wave must have been caused by a sudden addition to left atrial volume by the tumor returning from the left ventricle (8 9 36)

CONCLUSION

Non invasive techniques such as phonocardiography and echocardiography are very useful in establishing the diagnosis of left atrial myxoma However in a case of left atrial myxoma together with a large secundum type atrial septal defect it was not possible to detect this defect with the aim of these techiques alone Cardiac catheterization is necessary to exlude a shunt

REFERENCES

1 Adams C W Collins H A Dummit E S & Allen J H Intracardiac myxomas and thrombi Clinical manifestations pathology and treatment Am J Cardiol 7 176 1961

2 Aldridge H E & Greenwood W F Myxoma of the left atrium Br Heart J XXII 189 1960

3 Bahl O P Oliver G C Ferguson T B Schad N & Parker B M Recurrent left atrial myxoma Report of a case Circulation XL 673 1969

4 Beanlands D S Roy D L Dolan F G & Shane S J Myxoma of the left atrium Can Med Assoc J 83 715 1960

5 Boss J H & Bechar M Myxoma of the heart Report based on four cases Am J Cardiol 5 823 1959

6 Bower P J Ritter D G Callahan J A & Zitnik *R S Unusual hemodynamic findings of diagnostic* value in a case of left atrial myxoma Am J Cardiol 23 592 1969

7 Brown W O Myxoma of the heart Am Heart J 31 373 1946

8 van Buchem F S P & Eerland L D Myxoma cordis Diagnosis established pre-operatively surgical removal of the tumor Dis Chest 31 61 1957

9 van Buchem F S P Nieveen J & van der Slikke L B The diagnosis of myxoma cordis Diagnosis established pre-operatively in two cases Cardiologica 30 353 1957

10 Coates E O & Drake E H Myxoma of the right atrium with variable right to-left shunt N Engl J Med 259 165 1958

11 Cohen A I McIntosh H D & Orgain E S The mimetic nature of left atrial myxomas Am J Cardiol 11 802 1963

12 Craige E & Algary W P Left atrial myxoma Diagnosis with the help of the phonocardiogram and apexcardiogram Arch Intern Med 129 470 1972

13 Cumming G R & Finkel K Intracardiac myxoma involving the right and left atria in a young patient J Pediatr 58 559 1961

14 Farah M G Familial atrial myxoma Ann Intern Med 83 358 1975

15 Frankenfeld R H Waters C H & Steiner R C Bilateral myxomas of the heart Ann Intern Med 53 827 1960

16 Ghahramani A R Arnold J R Hildner F J Sommer L S & Samet P Left atrial myxoma. Hemodynamic and phonocardiographic features Am J Med 52 525 1972

17 Goldschlager A Popper R Goldschlager N Gerbode F & Prozan G Right atrial myxoma with right to left shunt and polycythemia presenting as congenital heart disease Am J Cardiol 30 82 1972

18 Goodwin J F Diagnosis of left atrial myxoma. Lancet 1 464 1963

19 Greenwood W F Profile of atrial myxoma Am J Cardiol 21 367 1968

20 Hair T E Orgain E S Sealy W C & McIntosh H D Myxoma of the left atrium Observations on two cases with succesful removal and review of diagnostic methods Am J Med 32 560 1962

21 Harvey W P Clinical aspects of cardiac tumors Am J Cardiol 21 328 1968

22 ten Hoor F Bepaling van de gemiddelde bloedstroomsterkte met indikatorverdunningsmethodes Groningen 1969

23 Kaufmann G Rutishauser W & Hegglin R Heart sounds in atrial tumors Am J Cardiol 8 350 1961

24 Lefcoe N M Brien F S & Manning G W An opening snap recorded in a case of tumor of the left atrium N Engl J Med 257 178 1957

25 Malloch C I Abbott J A & Rapaport E Left atrial myxoma with bacteremia. Report of a case with a bifid systolic apical impulse Am J Cardiol 25 353 1970

26 Malm J R Bowman F D & Henry J B Left atrial myxoma associated with an atrial septal defect J Thorac Cardiovasc Surg 45 490 1963

27 *Martin C E Hufnagel C A & de Leon A C* Calcified atrial myxoma diagnostic significance of the systolic tumor sound in a case presenting as tricuspid insufficiency Am Heart J 78 245 1969

28 McGregor G A & Cullen R A The syndrome of fever anemia and high sedimentation rate with an atrial myxoma Br Med J II 991 1959

29 Mook G A Directe oxymetrie tijdens hartcatheterisatie Groningen 1959

30 Nasser W K Davis R H Dillon J C Tavel M E Helmen C H Feigenbaum H & Fisch C Atrial myxoma I Clinical and pathologic features in nine cases Am Heart J 83 694 1972

31 — Atrial myxoma II Phonocardiographic echocardiographic hemodynamic and angiographic features in nine cases Am Heart J 83 810 1972

32 Nichols J & Hennigar G A case of ticentric cardiac myxoma 1959

tion time index was shortened as before 367 ms The Q(ECG) S I interval was now shorter, 60 ms

The apex cardiogram was unchanged there was a poor definition of the diastolic events an anacrotic notch at the moment of S I and a sharp systolic retraction with a midsystolic minimum

The postoperative echocardiogram showed a normal right ventricle and a paradoxical movement of the interventricular septum The anterior and posterior mitral valve leaflet showed a normal pattern with a normal E–F slope A small echo-free space between epicardium and pericardium was seen The left atrium was still slightly enlarged

DISCUSSION

The remarks in the literature on phonocardiographic signs in left atrial myxoma are not uniform This is not very surprising myxomas of different forms and weights in different hearts must give rise to different findings Mostly a loud prolonged S I was found sometimes an opening snap (24) and sometimes a so-called tumor plop The apex cardiogram mostly showed a prominent notch in the upstroke (12) corresponding to the loud S I

The S I was loud and prolonged in our patient there was a soft opening snap and also a tumor plop An early systolic sound coinciding with the deepest part of the tumor on the echocardiogram was also orded giving rise to the suspicion of an early tolic tumor sound

e splitting of S II might have been caused by the atrial sepatl defect and/or the left atrial myxoma In the case of a great left atrial myxoma there will be left atrial inflow obstruction of the pulmonary veins which causes splitting of S II (36)

After operation S I became softer the Q(ECG) S I interval became shorter the splitting of S II disappeared as did the opening snap The II P became soft The murmurs disappeared except for a very soft atriosystolic and an early systolic murmur

Echocardiography proved to be of great value a mass of echoes behind the anterior mitral valve leaflet is a characteristic pattern The E–F slope of this leaflet is diminished suggestive of mitral stenosis but the leaflet excursion is too large and the posterior leaflet is not visible

Many mistakes can be made insufficient rejection or damping or too high gain can give false positives too low gain false negatives A clot can mimic the picture which is why a sweep from the aorta to the left ventricular wall has also to be made

It is not always possible to perform a satisfactory registration due to deformity of the chest or because of pulmonary disease

Our patient showed a very clear echocardiogram the swinging of the left atrial myxoma could be seen from the left atrium to the left ventricle and back

During ventricular diastole, when the tumor has moved downward to the left ventricle only the smaller top of the tumor could be seen behind the aorta as a mass of echoes occupying about half of the left atrium During ventricular systole the tumor was seen in nearly the entire left atrium Directing the transducer downward the echoes filled up the mitral valve orifice also at the moment when the left ventricular posterior wall became visible which means that the tumor was in the left ventricular cavity at that moment After operation a normal pattern of the mitral valve was seen

The atrial septal defect could not be diagnosed by non invasive techniques despite the fact that there was a 36% left to right shunt One would expect a respiratory independent splitting of S II there was a splitting (that also could have been caused by the myxoma) but it was respiratory dependent The jugular venous tracing showed a normal aspect of *a* and *v* waves confirmed by the liver tracing In cases of atrial septal defect the *a* wave mostly measures less than 1 5×the *v* wave in this tracing this was more The direction of movement of the interventricular septum was normal before operation backwards during ventricular systole In cases of an atrial septal defect (right ventricular volume overload) a paradoxical movement might be expected The postoperative echocardiogram did show this but tricuspid insufficiency must have been the cause

Cardiac catheterization showed a higher oxygen saturation in the left atrium than in the vena cava The indirect left atrium pressure curve (wedge position) showed a small *a* wave, prominent *c* and *v* waves and a rapid *y* descent as described before (9 36) from direct left atrial pressure measurements by means of transseptal catheterization

The indirect pressure curve gives as much information as the direct one because of the risk of systemic embolization of the tumor a transseptal procedure should be avoided when the presence of a left atrial tumor is suspected (31)

Acquired Factor XII Deficiency in a Patient with Nephrotic Syndrome

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ABSTRACT A patient with nephrotic syndrome and an acquired factor XII deficiency associated with a factor XII like procoagulant activity in the urine was investigated The urinary protein with procoagulant activity was isolated and comparative investigations revealed similar properties to plasma factor XII It is suggested that the acquired coagulation defect may result from an insufficient biosynthetic capacity to compensate for the loss of factor XII in the urine

Key words factor XII nephrotic syndrome
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Deficiencies of coagulation factors are a potential risk of bleeding after kidney biopsy Acquired factor IX and factor XII deficiencies have been reported in nephrotic syndrome (3 4 5 11)

We describe a patient suffering from nephrotic syndrome with an acquired factor XII deficiency associated with a factor XII like procoagulant activity in the urine Results from studies on the protein with procoagulant activity isolated from the urine supported previous findings in which factor XII deficiency could be accounted for at least in part by loss in the urine

MATERIALS

Glassware was siliconized with a 1% silicone solution (Siliclad Clay Adams Parsippany New Yersey USA) No further reagents were used to inhibit contact activation of factor XII Urine from the patient was collected in siliconized bottles and stored at −30°C

METHODS

Gel filtration

Gel filtration was performed at room temperature using DEAE Sephadex A 50 and Sephadex G 100 (Pharmacia Fine Chemicals Uppsala Sweden) and eluted fractions were collected at 4 C DEAE Sephadex was equilibrated in 0 01 M sodium phosphate 0 001 M EDTA pH 8 0 and packed in a siliconized column (diameter 5 cm height 38 cm) A linear sodium chloride gradient was applied to the column with an elution rate of 1 5 ml/min two buffer reservoirs were filled one with 700 ml 0 01 M sodium phosphate 0 001 M EDTA pH 8 0 and the other with the same buffer containing 0 75 M sodium chloride Sephadex G 100 was equilibrated with 0 01 M sodium potassium phosphate 0 154 M sodium chloride pH 7 5 and packed in a siliconized column (diameter 2 5 cm height 90 cm) The equilibration buffer was also used as eluant with an elution rate of 1 0 1 2 ml/min

For the determination of the apparent molecular weight of the protein with factor XII-like procoagulant activity gel filtration was performed using Sephadex G 100 (column diameter 1 5 cm height 80 cm) with 0 01 M sodium phosphate buffer pH 7.5 as eluant

Coagulation assays

Factor XII activity was determined by a one stage clotting assay using human factor XII-deficient plasma (Dade Reagents Merz & Dade Bern Switzerland) In the assay 0 1 ml diluted urine (1 10 Michaelis buffer pH 7 4) was incubated with 0 1 ml factor XII-deficient plasma and 0 1 ml of a mixture containing 4 mg kaolin and 0 2 mg phospholipid/ml After incubation for 7 min at 37°C 0 1 ml 0 033 M $CaCl_2$ was added and the clotting time recorded Values are expressed in units (U) 1 U representing the procoagulant activity of 1 ml of normal human pool plasma (assayed in a 1 10 dilution)

Other coagulation factors were estimated by one stage clotting assays using human deficient substrate plasma as described previously (13) Antithrombin III was assayed by the method of Kahlé et al (7) using the chromogenic substrate Chromozym® TH on an AKES (Automated Kinetic Enzyme and Substrate Analyzer Vitatron Dieren The Netherlands)

Abbreviations BP=blood pressure BUN=blood urea nitrogen MW=molecular weight

Table I *Haemostatic data before and after therapy*

Method	Before therapy	After therapy	Normal value
Bleeding time (min)	1 5		1–7
Platelet count (×10⁹/l)	245	180	150–350
Prothrombin time (sec)	11 3	11 2	11 5
Partial thromboplastin time (sec)	120	45	35–63
Fibrinogen (g/l)	12 56	5 90	1 75–3 50
Factor II (U)	1 38	–	0 90–1 50
Factor V (U)	1 70	–	0 80–1 50
Factor VII (U)	1 70	–	0 70–1 60
Factor X (U)	1 30	–	0 80–1 40
Factor VIII (U)	1 70	1 58	0 50–1 50
Factor IX (U)	0 67	1 40	0 50–1 50
Factor XI (U)	0 64	1 80	0 40–1 60
Factor XII (U)	0 07	0 64	0 40–1 20
Antithrombin III (U)	0 32	1 10	0 80–1 20

CASE REPORT

A 23 year old man was admitted to the Juliana Hospital Amsterdam suffering from progressive oedema and proteinuria of more than 30 g/day Serum protein content was 30 g/l albumin 17 g/l with a marked lipaemia Blood urea nitrogen (BUN) and creatinine were within the normal range Investigations revealed that heavy metal intoxication amyloidosis bilharziosis and tuberculosis could be ruled out A kidney biopsy was scheduled and prebioptic screening of haemostatic functions revealed a marked prolongation of the kaolin-cephalin clotting time

At this time the patient was transferred to the Wilhelmina Gasthuis for further investigation Physical examination on admission revealed a blood pressure (BP) of 150/100 mmHg and a pulse rate of 68/min His body weight

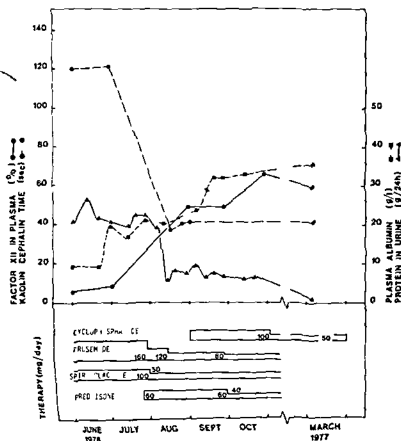


Fig 1 Plasma factor XII activity plasma albumin and proteinuria in the course of therapy

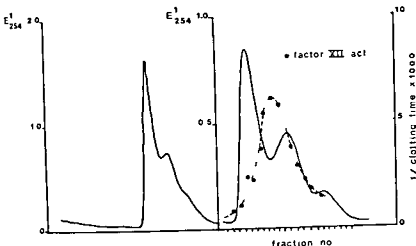


Fig 2 Elution profile of urinary factor XII following Sephadex G 100 recycling chromatography *Left* the elution pattern after the first chromatography cycle *right* (representing the second cycle) both the protein profile and the factor XII clotting activity

was 65 8 kg and height 1 64 m Facial oedema pleural fluid ascites and extensive pitting oedema of the legs were observed

Laboratory investigations of urine samples revealed that the sediment contained many hyalin casts with some leucocytes the protein concentration was 12 g/l (albumin 67 4% α_1 globulin 9 6% α_2-globulin 5 1% β-globulin 11 9% γ-globulin 6 0%) Fractional clearances of IgA and α_2-macroglobulin assuming a 100% clearance of transferrin were calculated to be 28 8 and 1 7% respectively indicating non selective proteinuria (6) Haematological screening revealed ESR 123 mm Hb 8 1 mM Hct 0 38 l/l leucocytes 16 3×10^9/l with a normal differential cell count Serum electrolytes BUN and creatinine were normal while total lipids (20 g/l) cholesterol (17 1 mM) and triglycerides (9 5 mM) were increased Serum proteins were decreased albumin 4 6 g/l α_1-globulin 1 2 g/l α_2 globulin 17 1 g/l β and γ globulin 7 2 g/l Liver enzymes and complement factors Clq C3 C4 and C5 were normal

Data from haemostatic investigations are given in Table 1 After infusion of 900 ml of fresh plasma the recovery of factor XII was calculated to be 38% of the administered dose The plasma half-life of factor XII procoagulant activity which was estimated by linear regression analysis of the factor XII procoagulant activities after infusion was 15 hours (normal value ca 60)

A kidney biopsy was performed without correction of the factor XII deficiency and no haemorrhagic complications were observed Light microscopy of the glomeruli revealed no cellular infiltration or proliferation and a normal basement membrane with no depositions while the tubuli were found to contain protein Immunofluorescence revealed no depositions of immunoglobulins or complement A diagnosis of nephrotic syndrome due to minimal lesions glomerulonephritis was made

Diuretic therapy with frusemide and spironolactone was instigated On this therapy the oedema slowly disappeared and the body weight decreased by 25 kg As proteinuria was not abated prednisolone was administered in a daily dose of 60 mg which decreased the excretion of protein from 25 g to 6 g/day Cyclophosphamide administered in a daily dose of 100 mg decreased proteinuria further to less than 1 5 g/day Plasma protein and factor XII procoagulant activity increased concurrently in the course of therapy (Fig 1)

After withdrawal of all therapy (April 1977) proteinuria remained at a level of ±1 5 g/day and the following coagulation values all within the normal range were found factor IX 1 4 U factor XI 1 8 U factor XII 0 64 U antithrombin III 1 1 U

RESULTS

Urine (protein concentration 12 g/l) when plasma factor XII procoagulant activity was 0 07–0 08 U and proteinuria exceeded 25 g/day contained no factor II/V/VII/X activity and 0 11 U factor VIII 0 01 U factor IX 0 01 U factor XI and 0 17 U factor XII like activity No such clotting activity could be demonstrated in normal human urine Incubation with kaolin significantly increased the procoagulant activity of the urine in the assay of factor XII Heating of the urine at 100°C for 10 min resulted in complete loss of the procoagulant activity Factor XII like procoagulant activity was concentrated by dialysis of the urine on a collodion membrane under negative pressure The procoagulant activity was almost completely absorbed by celite and following elution of the celite with 0 007 M NH_4OH pH 9 7 factor XII like procoagulant activity was found to be associated with the eluate Attempts were made to isolate the urinary protein with factor XII like procoagulant activity The patient s urine stored at −30 C served as starting material A cryoprecipitate which was found to have factor VIII procoagulant activity was formed during thawing at 4 C

After removal of the cryoprecipitate ammonium sulphate was added to the supernatant and the

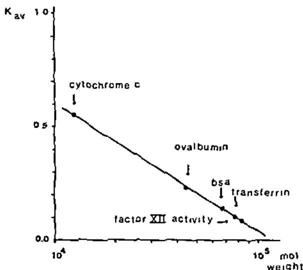


Fig 3 Determination of the apparent MW of urinary factor XII by a plot of elution volume versus log MW

factor XII like procoagulant activity was found to be associated with the precipitate formed at 25–50% saturation The precipitate was sedimented and dissolved in 0 154 M sodium chloride Following dialysis against 0 154 M sodium chloride the dialysate was applied to a DEAE Sephadex column Factor XII like procoagulant activity was eluted at an ionic strength of 0 150–0 165 Those fractions containing factor XII like procoagulant activity were pooled and concentrated by reprecipitation with ammonium sulphate (60% saturation) dialyzed against 0 154 M sodium chloride and applied to a Sephadex G 100 column A recycling procedure of the eluant from this column resulted in a better separation of the proteins Factor XII like procoagulant activity eluted just before the second protein peak (Fig 2)

Several batches of urinary factor XII like procoagulant activity were prepared by this method After concentration by negative pressure dialysis 11 preparations were pooled and submitted to rechromatography on Sephadex G 100 Fractions containing factor XII like procoagulant activity were pooled concentrated and used to determine the apparent molecular weight (MW) on a Sephadex G 100 column The column was equilibrated with transferrin (MW 77000) bovine serum albumin (MW 67000) ovalbumin (MW 45000) cytochrome c (MW 12500) The void volume was determined using dextran blue and the total volume of the gel bed by phenol Factor XII like procoagulant activity was eluted at a volume corresponding to an apparent MW of 82000 daltons (Fig 3)

The fractional clearance of factor XII relative to the transferrin clearance was 57% Assuming an MW of 82000 daltons for factor XII, the data obtained are in close agreement with the curve for the fractional clearance of IgA and α_2 macroglobulin (Fig 4) This suggests that there was no preferential loss of factor XII compared to other proteins

DISCUSSION

Coagulation and haemostasis investigations on patients with nephrotic syndrome have revealed disparate results Increase in platelet count fibrinogen level and factor VIII procoagulant activity have been observed in association with this syndrome (8 10) However coagulation factor deficiencies have also been observed in some patients A deficiency of antithrombin III in nephrotic syndrome resulting in a thrombotic tendency has been described by Kaufmann et al (9), while Handley and Lawrence (4) described a haemorrhagic diathesis associated with decreased levels of factor IX Factor XII deficiency is not in general associated with a bleeding tendency but does result in a prolongation of the kaolin cephalin clotting time and has also

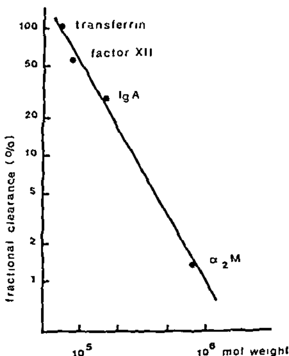


Fig 4 Fractional clearance of factor XII

been reported to occur in nephrotic syndrome (3 5)

We present a patient with low levels of factor XII and antithrombin III on whom a kidney biopsy was performed without haemorrhagic complications although the factor XII deficiency had not been corrected Decreased factor XII procoagulant activity in association with a markedly shortened plasma half life together with a severe proteinuria were indicative of factor XII loss in the urine In the course of therapy the low factor XII and antithrombin III levels were normalized parallel with the increase in plasma albumin

Antithrombin III antigen and a procoagulant activity which have been found to induce antibodies against human factor IX in rabbits have been found in the urine of patients with nephrotic syndrome (9 11) Green et al (3) described a patient with low levels of factors IX and XII They demonstrated factor XII like procoagulant activity in the urine which showed the same elution profile as plasma factor XII when filtered through Sephadex G 100 However no attempts were made to isolate the active protein Factor XII like procoagulant activity was also demonstrated in the urine of our patient The factor XII like material was labile to heat could be absorbed to and eluted from celite and was activated by kaolin In addition the elution profile after filtration through DEAE Sephadex was similar to that of plasma factor XII (2) Following further purification the active molecule was found to have an apparent MW of 82000 daltons which is in agreement with quoted values for plasma factor XII (12)

A proper explanation for the deficiency of antithrombin III and of factor XII in this patient is not yet apparent The MWs of antithrombin III and factors II VII X and XII are within the range of 40000–80000 daltons therefore MW cannot be the sole determinant of the selective loss of antithrombin III and factor XII

Differences in biosynthetic capacity as well as in molecular charge (1) of the various clotting factors may determine their plasma level in proteinuria In our patient the loss of factor XII protein also revealed by the markedly shortened half life appeared to be proportional to that of other proteins such as IgA α_2-macroglobulin since the plot of log MW against the fractional clearance of factor XII fitted the curve obtained for transferrin IgA and α_2-macroglobulin

These findings strongly suggest that the factor XII deficiency in this patient resulted from an insufficient biosynthetic capacity of this protein relative to other clotting factors to compensate for the loss in the urine A similar explanation may account for the deficiency of antithrombin III and the plasma factor VIII procoagulant activity of 1 7 U which is low for a patient with nephrotic syndrome especially in view of the high plasma fibrinogen level

REFERENCES

1 Brenner B M Hostetter T H & Humes H D Molecular basis of proteinuria of glomerular origin N Engl J Med 298 826 1978
2 Cochrane C G & Wuepper K D The first component of the kinin forming system in human and rabbit plasma Its relationship to clotting factor XII (Hageman factor) J Exp Med 134 986 1971
3 Green D Arruda J Honig G & Muercke R C Urinary loss of clotting factors due to hereditary membranous glomerulopathy Am J Clin Pathol 65 376 1976
4 Handley D A & Lawrence J R Factor IX deficiency in the nephrotic syndrome Lancet 1 1079 1967
5 Honig G R & Indley A Deficiency of Hageman factor (factor XII) in patients with the nephrotic syndrome J Pediatr 78 633 1971
6 Joachim G R Cameron J S Schwartz M & Becker E L Selectivity of protein excretion in patients with the nephrotic syndrome J Clin Invest 43 2332 1964
7 Kahle L H Schipper H G Jenkins C S P & ten Cate J W Antithrombin III I Evaluation of an automated antithrombin III method Thromb Res 12 1003 1978
8 Kanfer A Kleinknecht D Broyer M & Josso E Coagulation studies in 45 cases of nephrotic syndrome without uremia Thromb Diath Haemorrh (Stuttg) 24 562 1970
9 Kaufmann R H de Graeff J Brutel de la Riviere G & van Es L A Unilateral renal vein thrombosis and nephrotic syndrome Report of a case with protein selectivity and antithrombin III clearance studies Am J Med 60 1048 1976
10 Kendall A G Lohmann R C & Dossetor J B Nephrotic syndrome a hypercoagulable state Arch Intern Med 127 1021 1971
11 Natelson E A Lynch E C Hettig R A & Alfrey C P Acquired factor IX deficiency in the nephrotic syndrome Ann Intern Med 73 373 1970
12 Revak S D Cochrane C G Johnston A R & Hugli T E Structural changes accompanying enzymatic activation of human Hageman factor J Clin Invest 54 619 1974
13 van Royen E A Treffers P E & ten Cate J W Hypertonic saline induced abortion as pathophysiologic model of low grade intravascular coagulation Scand J Haematol 13 166 1974



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Compression of the Inferior Caval Vein— A Rare Complication of a Large Non-Parasitic Liver Cyst

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ABSTRACT A patient with massive edema of the legs and scrotum is presented in whom non parasitic cysts of the liver were found Surgical evacuation of one large lobulated liver cyst completely relieved the patient of his edema It is concluded that in this patient the peripheral edema was caused solely by cystic obstruction of the inferior caval vein

Key words non parasitic liver cyst inferior caval vein peripheral edema

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Solitary or multiple non parasitic cysts of the liver are relatively rare and usually asymptomatic Nevertheless rupture of a cyst into the peritoneal cavity (2 8 10 11) strangulation (5 12) torsion (13) intracystic hemorrhage (1 5) jaundice caused by obstruction of extrahepatic ducts (3 7 9) and various abdominal symptoms due to pressure on some part of the gastrointestinal tract (4 6 8 11) have been reported However patients with non parasitic liver cysts presenting as the only clinical sign pitting edema of the lower limbs and scrotum have to the best of our knowledge not been described before In this paper we will report on such a patient

CASE REPORT

A previously healthy man aged 77 was brought to hospital because of a traffic accident On admission a large rounded smooth painless mass was palpated in the right upper abdominal quadrant Moreover a fresh supracondylar fracture of the left femur was found and conservative treatment with traction and immobilization and supine bed rest was applied for two months In order to prevent intravascular clotting during this period an oral anticoagulant (warfarin) was given Six weeks after admission ankle edema appeared showing gradual and similar progression on both sides In view of the progressive peripheral edema it was considered hazardous to continue with the conservative treatment and osteosynthesis (Rush pin) was therefore carried out. Although this enabled mobilization of the patient the peripheral edema continued to progress One month after the operation both legs and scrotum were massively swollen At that time the patient was transferred to a medical ward for further examination

Examination in the medical ward disclosed no physical signs indicating cardiac disease except for pitting edema below the umbilical level BP was normal (130/70 mmHg) pulse rate 64/min Chest X ray revealed a heart volume of 520 ml/m² and pulmonary vessels of normal size The painless abdominal mass approximately 150 mm in diameter was still palpable in the right upper abdominal quadrant but there were no other clinical or laboratory signs indicating hepatic cirrhosis or gross impairment of the liver function Serum albumin was 40 g/1 000 ml Proteinuria was never found When percutaneous phlebography of the inferior caval vein was carried out in order to exclude caval thrombosis a tapering obstruction of the vein was found (Fig 1) located at the same level as the intra abdominal mass Subsequent percutaneous selective hepatic angiography revealed a large hepatic avascular mass probably of cystic nature Computerized axial tomography (Fig 2) confirmed the diagnosis One large cyst displacing adjacent intra abdominal organs was located within the right hepatic lobe and three smaller cysts were found within the left lobe No cysts were detected in the spleen or in the kidneys

Having ruled out Echinococcus infection by serological tests the patient was laparotomized On opening the abdomen a large lobulated liver cyst was found The cyst was drained and found to contain 2 800 ml of brownish fluid Microscopy of the cyst wall showed it to be lined by a single layer of cuboid epithelial cells Postoperatively the edema rapidly vanished and two months after the surgical evacuation the peripheral edema still showed no tendency to recur

DISCUSSION

Most non parasitic liver cysts do not give rise to clinical symptoms Our patient showing a huge intrahepatic cyst compressing the inferior caval vein did not complain of abdominal fullness or distress either Nevertheless the cyst contained a brownis



Fig 1 Phlebography of the inferior caval vein A tapering occlusion is noted in the liver region (arrows) Widened collaterals in the lumbar region

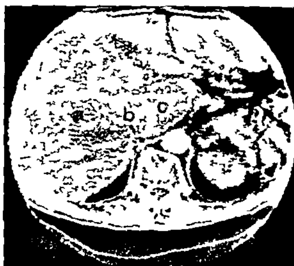


Fig 2 CAT scan through the lower part of the liver a = Area of reduced attenuation representing a large cyst in the right liver lobe b = The compressed inferior caval vein c A small cyst A scan 40 mm more cranially showed multiple small cysts in the left liver lobe

fluid when evacuated several months after the traffic accident Thus an intracystic bleeding resulting in an increased cyst volume might have occurred either in connection with the traffic accident or later A spontaneous intracystic hemorrhage occurring some time during the period of an lant treatment seems most likely inasmuch the ankle edema did not appear until several s after the traffic accident Confinement to bed might also have contributed to the caval compression because the cyst was overriding the inferior caval vein when the patient was resting in the supine position It must be emphasized however that immobilization cannot have been the sole explanation for the caval compression since the edema continued to increase even after mobilization of the patient and did not disappear until surgical evacuation of the large liver cyst had been carried out

REFERENCES

1 Ackman F D & Rhea L J Non parasitic cysts of the liver Their clinical and pathological aspects Br J Surg 18 642 1931

2 Brunes L Rupture of a solitary nonparasitic cyst of the liver Report of a case Acta Chir Scand 140 159 1974

3 Caravati C M Watts T D Hopkins J E T & Kelly F R Benign solitary non parasitic cyst of the liver Gastroenterology 14 317 1950

4 Geist D C Solitary nonparasitic cyst of the liver Review of the literature and report of two cases Arch Surg 71 867 1955

5 Grime R T Moore T Nicholson A & Whitehead R Cyst haematomas and polycystic disease of the liver Br J Surg 47 307 1959

6 Hadad A R Westbrook K C Graham G G Morris W D & Campbell G S Symptomatic nonparasitic liver cysts Am J Surg 134 739 1977

7 Hallenbeck G A & Fricke R W Traumatic bile cyst of liver report of two cases Mayo Clin Proc 25 648 1950

8 Horton R E Giant cyst of the liver complicated by rupture Br J Surg 41 442 1953

9 Lloyd Jones W Mountain J C & Warren K W Symptomatic non parasitic cysts of the liver Br J Surg 61 118 1974

10 Lulenski C R Rupture of solitary cyst of the liver Ohio State Med J 44 874 1948

11 Morgenstern L Rupture of solitary non parasitic cysts of the liver Ann Surg 150 167 1959

12 Orr T G & Thurston J A Strangulated nonparasitic cyst of the liver Ann Surg 86 901 19 7

13 Sood S C & Watson A Solitary cyst of the liver presenting as an abdominal emergency Postgrad Med J 50 48 1974

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Why Do Crystalline Precipitates in Plasma Cells always Coalesce with Parallel Light and Dark Lines Strictly End-to-End and Side-to-Side?

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ABSTRACT During transmission electron microscopy of plasma cells from two patients with plasma cell proliferative disorders, we observed a large number of small crystals, most of which showed a striation with alternating light and dark parallel lines Ultraphotos strongly suggested that smaller crystals *often coalesced to larger ones* The coalescence occurred in such a way that the parallel lines of the respective smaller crystals always met each other end to-end and hardly ever end to-side A similar pattern can be seen on previously published ultraphotos from other patients with plasma cell proliferative disorders and intracellular crystals although not commented upon by the respective authors The parallel lines of the smaller crystals certainly must correspond to defined structures of the crystal The stability of a certain configuration is therefore in all likelihood due to electrical potentials created by the crystal structure

Key words plasma cells crystals—configuration of
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Crystals are occasionally found in the plasma cells in plasma cell proliferative disorders Sometimes they are located in the cisternae of the rough endoplasmic reticulum (RER) like crystalline Russel bodies (1) At other times the crystals are located in the cytoplasm outside the RER (1 2 3 4 5) either enclosed in a smooth membrane which might derive from the Golgi apparatus or without such a membrane

OWN OBSERVATIONS

We have recently studied two patients with plasma cell proliferative disorders who had a large number of crystals in their plasma cells Transmission electron microscopy of glutaraldehyde fixed and osmium tetroxide post fixed lead citrate and uranyl stained plasma cells showed that most of the small crystals had a striation with alternating light and dark parallel lines

A large number of ultraphotos of these plasma cells strongly suggested that smaller crystals would coalesce to larger ones The coalescence occurred in such a way that the parallel lines of the smaller crystals always met each other end to-end and hardly ever end to-side Fig 1 illustrates this rather well The orderly pattern with parallel dark and light lines is clearly visible This ultraphoto shows coalescences of crystalline precipitates in which parallel lines of the respective smaller crystals meet each other at a certain angle always end to-end A *coalescence in which the parallel lines of the re*spective smaller precipitates meet each other end to-end in *almost* perfect alignment is also apparent in Fig 1 as well as a large crystal showing perfect longitudinal striation throughout *If* this crystal has been produced by coalescence of smaller ones they must have achieved perfect end to-end and side to-side alignment A look at some of the ultraphotos in previous publications from other patients with plasma cell proliferative disorders (3 4) reveals the same end to-end pattern although not commented upon by the authors

If the smaller crystals had been located in the cisternae a longitudinal axis tangential to the surface of the nucleus might have prevailed making fusion with the parallel lines end to-end natural However the crystals in our two patients are *not* located inside the RER and as Fig 1 illustrates the longitudinal axis of a large crystal can point in almost any direction When the smaller crystalline precipitates nevertheless fuse with the parallel lines strictly end to-end and side to-side the reason must be that this configuration is stable The p
l f the smaller crystals certainly

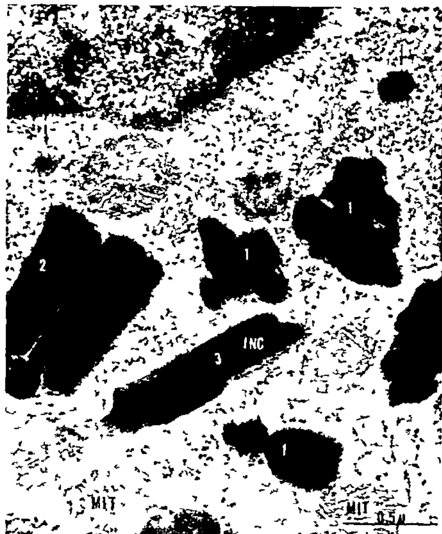


Fig 1 Arrows show earliest crystalline precipitations *1* = Coalescence of smaller crystalline precipitates with parallel lines roughly but not perfectly end to-end *2* = Coalescence of crystalline precipitates with parallel lines almost perfectly end to-end *3* = A fairly large crystal in which smaller fragments are not distinguishable Two of the 5 mitochondriae are marked with *MIT* The line in the right lower corner is 0 5 μ long

respond to defined structures of the crystal The stability of the end to-end configuration is therefore in all likelihood due to electrical potentials created by the crystal structure

If the in vivo development of hundreds of smaller and larger crystals inside of plasma cells differs from the usual concept of in vitro crystallization from organic and inorganic solutions the main reason for the difference might lie in the structure and composition of the cytoplasm The various precipitates and their coalescence to larger precipitates in the plasma cells might then perhaps afford a useful model for studying certain aspects of crystal formation in general

ACKNOWLEDGEMENT

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REFERENCES

1 Bessis M Living blood cells and their ultrastructure pp 767 Springer Berlin 1976
2 Cordier A C Scolari L. Vaerman J P Dutrieux Fauchet M-C Dutrieux C & Haumont S Ultrasctructural aspects of crystal like inclusions in a case of IgA plasma cell proliferation Scand J Haematol 17 143 1976
3 Maldonado J E Velosa J A Kyle R A Wagoner R D Holley K E & Salassa R M Fanconi syndrome in adults Am J Med 58 354 1975
4 Oikawa K Electron microscopic observation of inclusion bodies in plasma cells of multiple myeloma and Waldenström s macroglobulinemia Tohoku J Exp Med 117 257 1975
5 Stavem P Vandvik B Skrede S & Hovig T Needle like crystals in plasma cells in a patient with a plasma cell proliferative disorder Scand J Haematol 14 23 1975

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EDITOR'S INTRODUCTION

The dream of every editor is to shorten to a minimum the publication time of a paper from acceptance to appearance in print Many people would think that this is easy and depends upon good organization in the offices of the editor and of the printer This is of course of paramount importance but there are many possible delays en route The ideal would be to reject a high percentage of papers and only print the very best This sounds good but in real life there are many other considerations Very few papers are all bad or completely good and the weighing is difficult We have very great help from the referees but it is clear to us that some of them look with a lenient eye even on rather mediocre papers whereas others have a hawk s eye for every fault In the big countries the offer of papers is much more varied The fact that many papers are in a rather poor shape when they are presented to the journal causes considerable delay The correction of the language and the editing of spelling takes a considerable time also in first-class papers We shall be much more severe in our criticism of second rate editing before a paper is submitted to the Acta This will save time for all papers in that number

In order to speed up the flow of papers we have decided to publish this extra number that was edited by a special person who was chosen for that job We hope that this will meet approval from authors whose papers will appear more rapidly It is also our hope that numbers devoted to special subjects will be popular among the readers One of the advantages of a journal like the Acta Medica Scandinavica is the fact that it embraces all specialities in internal medicine and thus represents general medicine and not just a specialty On the other hand we have many papers that belong to one special subgroup in medicine This number on diseases of the kidney is an example

Jan G Waldenström

BOOK REVIEWS

Clinical Nephrology 2nd ed By S Papper 633 pages $22 50 Little Brown and Co Boston 1978

It is a rare event nowadays to find a textbook of this size written entirely by one single author Dr Papper has now been able to revise and update his book from 1971 The new edition will undoubtly be one of the most used texts in this field for the next five years or more

This book has some of the disadvantages of a single authored book but all the advantages and charm too It is easy to enumerate mistakes in the text but they are not of grave consequence The discussions of controversial questions often extensive and generally well balanced sometimes favour a point of view that today seems dated It is an impossible task to have a critical opinion on the latest developments in every area of this rapidly developing specialty

The discussion of asymptomatic bacteriuria and of the methods to define the level of the infection of the urinary tract are examples of this

Nephrology even with the prefix "clinical" draws heavily from other fields of medical science in particular pathology immunology and physiology It remains a difficult problem in a textbook of this scope to decide how much to include from these fundamental areas Dr Papper has chosen to use only a rather small amount of pathology and even less of immunology in support of his explanation of the clinical problems Particularly the chapters on glomerular diseases and the nephrotic syndrome—the most difficult ones to write and to understand in any book on nephrology—must be hard to assimilate without some previous knowledge of glomerular pathology It is more difficult to understand why the important problems of the pathogenesis of glomerular diseases immunological or not are only hinted at The physiology on the other hand is well used in the excellent chapter on "Structure and function of the kidney"

The treatise carries the flavour of a specialized university hospital department to a sometimes disturbing degree There is very little discussion on when and how to work up an individual patient. When such advice is given as in the section on hypertension it would be impossible to implement in general practice There is very little discussion on the treatment of urinary tract infection (but asymptomatic bacteriuria should be treated!) The otherwise extensive and useful index carries one sole entry under "Cystitis"—honeymoon cystitis! In other areas I feel that it is a strength in this book that definite advice on how to handle certain problems is avoided Dr Papper repeatedly emphasizes that we should not investigate a certain symptom or sign such as haematuria, but assess a problem in an individual person However the chapter "Evaluation of treatment" gives some helpful hints to the management of certain problems although the advice on the testing of proteinuria or haematuria is unnecessarily complicated and the discussion on radiography of the kidney is so superficial that it might as well have been left out The methods recommended for the measurement of proteinuria or albuminuria (the distinction is not discussed) or of glomerular filtration rate would be regarded as both oldfashioned and unsatisfactory on this side of the Atlantic The same applies to the scant presentation of the uses of radioisotopes ultrasound or computerized body scanning The same slightly oldfashioned and unpractical attitude is particularly evident in the appended Handbook that describes certain test and methods used in nephrology

It is easy to find faults in this book but even easier to make friends with it The book is eminently readable partly because of its relatively low "information density"—facts per page—but mostly because of clarity of language and the consistency in style and method of presentation which is one of the major advantages of a textbook written by a single author

The greatest virtue of this type of books for students and young doctors of today is that it gives a natural impetus to critical reading Dr Papper constantly refers to opinions and interpretations of the literature as being only his own The pronouns I and me are used throughout the book No houseofficer would be tempted to quote these opinions of Dr Papper s as latter-days dogmas but only as "Dr Papper says It is a great step forward from the pretense of omniscience in many of the presently used textbooks—inside and outside nephrology—with five authors per chapter and a barrage of references in the text

The book is "written with the non nephrologist in mind" If there is one single book to be recommended to the future specialist in e g internal medicine or maybe urology it is this one It is a good introduction also for the budding nephrologist There is an extensive a la carte list of selected reading well chosen at the end of each chapter and it should be used especially to find additional information on renal pathology and on the pathogenesis of glomerulopathies In spite of its size the book can be read almost from cover to cover faster than many smaller books (excluding the "Handbook" at the end) and it will give much more information and pleasure too

The clinical wisdom of Dr Papper often illuminates the discourse Thus in the discussion of the treatment of a patient in chronic renal failure there is a section on "Sources of pleasure" "Every patient I see is asked What do you do for fun?" It is amazing how many (including those who are not chronically or seriously ill) respond with astonishment or with some remark suggesting that this dirty three letter word is not part of their lives "

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Practical Diagnosis/Renal Disease By M A Kirschenbaum 253 pages Houghton Mifflin Professional Publishers Boston 1978

The laboratory tests used in evaluating patients with renal disease or electrolyte disorders are numerous and varied This book is intended to serve as a guide to medical students house officers and practitioners in selecting and interpreting these tests"

So far so good What else do medical students read? If he—or she referring to the occasional majority these days—has learnt her physiology pathology microbiology and immunology out of decent text books and something about renal diseases and disturbances of renal function out of a book on internal medicine then this pocket size book could help to integrate this knowledge in a clinical setting. But the book goes beyond its proclaimed purpose and discusses briefly but superficially most areas pertinent to nephrology The temptation is to use the book as a syllabus on renal diseases As such it is not bad There is for instance an excellent table on pathological findings in glomerular diseases Four procedures for testing the concentration function are described including the nicotine test

The book can be and will be used by students as a quick and easy way to learn the right answers in a multiple choice examination on renal diseases Whether they will learn anything else from it depends on their background

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Renin–Angiotensin System in Mild Essential Hypertension

The Functional Significance of Angiotensin II in Untreated and Thiazide Treated Hypertensive Patients

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ABSTRACT Twenty five 40-year old patients with mild essential hypertension, identified during a survey of a population born in 1936 were investigated Basal and postfurosemide values for plasma renin concentration (PRC) and plasma angiotensin II concentration (PA II) did not differ markedly from reference values measured in 40 year-old control subjects from the same population In the untreated sodium replete state, saralasin infusion (5 4 nmol/kg/min) produced an increase in mean arterial pressure (MAP) in the patient group as a whole Twenty one patients were treated with hydrochlorothiazide, mean dose 75 mg/day for 3 months, and the examinations were repeated in 20 patients Pretreatment *furosemide stimulated PRC and PA II and* values during thiazide treatment were higher in ' non responders ' to hydrochlorothiazide treatment ($n=10$) than in ' thiazide reponders" ($n=11$) During thiazide therapy, angiotensin II blockade induced a clear cut decrease in MAP in *every single* ' thiazide non responder , whereas 7 out of 11 ' thiazide responders' did not show any fall in BP, and 4 exhibited only a borderline decline in MAP After thiazide *and* angiotensin II blockade, significant differences in MAP *were no longer present between the two* patient groups It is concluded that in mild *untreated* essential hypertension, angiotensin II has no decisive role in the maintenance of high BP The functional significance of the renin–angiotensin system (RAS) emerges *after thiazide* treatment Thiazide-induced stimulation of RAS coutnerbalances the hypotensive effect of thiazide in some 40% of the treated patients Thus the responsiveness of RAS determines the quantitative BP response to treatment Assessment of angio*tensin II response to acute stimulation could to some* extent predict the subsequent response to thiazide treatment Saralasin infusion serves as a reliable tool for assessing the participation of angiotensin II in BP regulation

Key words angiotensin II angiotensin II antagonist hypertension
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Measurements of plasma renin activity (PRA) in blood samples from patients with essential hypertension and normotensive controls have led to classification of patients with essential hypertension in to renin subgroups (13 23) This approach per se has not settled the role of angiotensin II in the maintenance of hypertension By means of specific competitive inhibitors of angiotensin II e g saralasin it is now possible to assess the participation of *angiotensin II in hypertension more directly* (5 6 28)

A substantial fraction of patients with mild to moderate essential hypertension respond satisfactorily to thiazide treatment although adequate blood pressure (BP) control is not achieved in all The activation of the renin–angiotensin system (RAS) during thiazide treatment has been claimed to be the mechanism responsible for the unsuccess*ful outcome of thiazide treatment* in some patients (12 23 29 32) This hypothesis is supported by the demonstration of high renin values during thiazide treatment but for a further clarification an assessment of the functional significance of raised renin or angiotensin II values is necessary e g by means of angiotensin II blockade

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Abbreviations *PRC* = plasma renin concentration PA II = plasma angiotensin II concentration MAP = mean arterial pressure BP = blood pressure RAS = renin–angiotensin system Na_E = exchangeable sodium PV = plasma volume PAC = plasma aldosterone concentration PNC = plasma noradrenaline concentration CEI = converting enzyme inhibitor

The aim of the present study has been to investigate by means of saralasin the functional involvement of RAS in the maintenance of high BP in a homogeneous group of patients with mild essential hypertension in the untreated state as well as during thiazide treatment

SUBJECTS

Twenty five patients (7 ♀ 18 ♂) with essential hypertension (WHO I) identified during a survey of a population born in 1936 were investigated None of them had ever received antihypertensive treatment Diastolic BP (phase V) as measured in the Outpatient Clinic after 10 min of rest in the supine position was 95 mmHg or more on 3 occasions Secondary hypertension was excluded by an examination programme including physical examination measurements of serum electrolytes and plasma noradrenaline/adrenaline concentration together with radioisotope renography Urography and/or angiography was carried out when renography showed abnormalities All patients had normal heart size as determined by chest X-ray and normal ECG 13 had retinal changes grade I (Keith Wagener Barker scale) and 12 presented with normal fundi Renal function as assessed from measurements of [51]Cr EDTA clearance was normal in all patients

Twenty five 40-year-old normotensive individuals (10 ♀ 15 ♂) selected from the study population served as a control group

The examination programme and the aim of the study were explained in detail and consent was obtained in all cases

In the sodium replete state 24-hour urines were collected exchangeable sodium (Na_E) was determined and plasma volume (PV) was measured Blood samples for measurements of plasma renin concentration (PRC) plasma aldosterone concentration (PAC) plasma angiotensin II concentration (PA II) and plasma noradrenaline concentration (PNC) were collected at rest in the supine position at 10 a m and after i v administration of furosemide 0 65 mg/kg b wt with subsequent quiet ambulation for 2 hours

On another day a saralasin infusion was given to the hypertensive subjects without any interference with sodium balance prior to infusion After 60 min rest in the supine position an infusion of glucose 5% was given for 30 min followed by a graded infusion of saralasin for 35 min in the supine position The rate of infusion was 0 54 nmol/kg/min for 10 min 2 7 for 10 min and 5 4 for 15 min An infusion pump (B Braun Melsungen Unita I) was used Saralasin was supplied by Dr M C F Cox Eaton Laboratories Norwich NY USA

BP (arteriosonde 1217) was recorded at 2 min intervals during the control period of 30 min during the 35 min of saralasin infusion and during 30 min after infusion Mean arterial pressure (MAP) was calculated as diastolic BP plus 1/3 of the amplitude The control "presaralasin" MAP was calculated as the average of the last three measurements over a 6 min period before saralasin infusion MAP during saralasin infusion was determined as the mean of the last 3 measurements before cessation of the infusion or the mean of 3 consecutive measurements with a lower BP level in any other period during the infusion at a rate of 5 4 nmol/kg/min Similarly the postinfusion BP level was defined as the mean of the last 3 measurements after discontinuation of saralasin infusion An analysis of the variance of MAP measurement in all presaralasin" periods showed that a change in mean MAP of 5 mmHg or more in a given individual was significant ($p<0\ 01$ two-tailed) A transitory decrease of 5 mmHg or more at the infusion rate of 5 4 nmol/kg/min with a return towards control level before discontinuation of the infusion was designated borderline response

Blood samples for measurements of PRC PAC PNC and plasma saralasin concentrations were collected at the end of the control period before starting saralasin infusion and at the end of the infusion period Samples for measurement of PA II were drawn just before the start of saralasin infusion (16)

Out of the 25 patients 21 were treated with hydrochlorothiazide 50–100 mg/day (mean 75) After 3 months of treatment the examinations were repeated in 20 patients

Na_E was measured with [24]Na using an equilibration period of 24 hours A correction for renal loss of [24]Na was applied PV was determined with [125]I-albumin (18) PRC and PA II were measured as previously described (14 19) PAC was measured by a modification of the method of Mayes et al (25) Our method for measuring plasma saralasin concentration has recently been published (20) PNC was measured as described by Christensen (9)

The statistical calculations were carried out by means of Wilcoxon s test for paired differences and the Mann Whitney rank sum test for unpaired observations Results are presented as median values with range in parentheses

RESULTS

The PRC PA II and PAC values supine at rest did not differ between hypertensive patients and controls (Table I) Values after acute stimulation were also nearly identical Similarly PNC values did not differ

Before treatment PV/m² BSA tended to be lower in hypertensive men and women than in normotensive controls (Table II) When expressed as percent of the values for 40-year-old normal individuals PV was 92 8% (72 9–119 4) as a group median this value was significantly lower ($p<0\ 05$) than 100% Na_E was identical in hypertensive and normotensive individuals (Table II)

Changes in BP during thiazide treatment are given in Table III Taking a fall in diastolic BP of ≥10 mmHg across thiazide treatment as the criterion the patient group was divided into 11 thiazide responders and 10 thiazide non responders

In the untreated sodium replete state saralasin infusion caused a small but significant increase in MAP from 108 (94–135) to 113 mmHg (94–135)

Table I *PRC PA II PAC and PNC at rest supine (A) and after furosemide + ambulation (B) in patients with mild untreated essential hypertension and normotensive controls (median values with range in parentheses)*

	Patients (n=23)	p	Controls (n=25)
PRC (mIU/l)			
A	29 (11–67)	n s	31 (11–49)
B	57 (14–162)	n s	78 (17–225)
PA II (pmol/l)			
A	8 (4–22)	n s	10 (4–20)
B	26 (6–72)	<0 05	35 (1?–91)
PAC (pmol/l)			
A	195 (56–417)	n s	167 (111–361)
B	556 (56–1446)	n s	667 (222–2029)
PNC (nmol/l)			
A	1 27 (0 51–2 04)	n s	1 21 (0 64–2 29)
B	2 36 (0 96–3.50)	n s	2 36 (1 46–4 01)

(p<0 05) In 6 out of the 25 patients the individual increase was significant Only 2 out of the 25 patients displayed a significant decrease in MAP Changes in MAP were similar in thiazide responders and thiazide non responders PAC increased by 126% (p<0 01) across saralasin infusion PRC decreased by 8% (n s) and PNC showed an insignificant decrease of about 20%

Whereas supine resting pretreatment values of PRC and PA II did not differ between the two groups of patients pretreatment furosemide stimulated PRC was 84 mIU/l (42–162) in thiazide non-responders (n=10) and 41 mIU/l (14–106) in responders (n=9) (p<0 05) (Fig 1) Similarly PA II was 37 pmol/l (range 12–72 n=10) and 14 pmol/l (range 6–32 n=8) (p<0 05) in non responders and responders respectively after acute stimulation (Fig 2) Furthermore the absolute increase in PA II from resting to acutely stimulated values was much greater in non responders 29 pmol/l (11–52) than in responders 9 pmol/l (2–19) (p<0 01) Neither resting nor acutely stimulated PAC differed between the two groups

PV and Na_E tended to be lower in non responders than in responders before as well as during thiazide treatment the differences were not statistically significant PV decreased significantly (p<0 05) during thiazide treatment (4%) Na_E did not change significantly The changes in PV and Na_E were similar in thiazide responders and non responders

Treatment caused a significant (p<0 01) about 2–3 fold increase in PRC and PA II (Figs 1 and 2) Postthiazide PRC was higher in thiazide non responders (n=9) 96 mIU/l (17–262) than in responders (n=11) 49 mIU/l (35–142) (p<0 02) Similarly PA II during treatment was 37 pmol/l (23–61) in non responders (n=8) and 26 pmol/l (4–41) in responders (n=11) (p<0 05) The increase in PA II from the untreated to the thiazide treated state was 28 pmol/l (15–55) in non responders and 16 pmol/l (1–27) in responders

Table II *PV and Na_E in patients with mild untreated essential hypertension and normotensive controls (median values with range in parentheses)*

		n	Patients	p	n	Controls
PV (ml/m² BSA)	Males	18	1557 (1223–2004)	n s	15	1718 (1341–1977)
	Females	6	1460 (1379–1551)	n s	10	1593 (1388–1863)
PV (% of normal)		24	92 8 (72 9–119 4)	<0 05	25	100 3 (80 0–117 9)
Na_E (mEq/m² BSA)	Males	18	1522 (1398–1937)	n s	14	1543 (1311–1850)
	Females	6	1398 (1335–1591)	n s	10	1400 (1233–1582)
Na_E (% of normal)		24	98 2 (87 9–121 8)	n s	24	99 8 (82.5–116 4)

Table III *Changes in BP (mmHg) during 3 months of hydrochlorothiazide treatment*
Mean dose 75 mg/day (median values with range in parentheses)

	n	Untreated		On thiazide	
		Systolic	Diastolic	Systolic	Diastolic
Total group	21	148 (131–184)	104 (95–113)	140 (120–170)	95 (84–112)
Thiazide responders	11	151 (131–184)	106 (98–113)	134 (120–145)	92 (84–98)
Thiazide non-responders	10	147 (131–184)	103 (95–111)	148 (125–170)	99 (91–112)

($p<0.01$) PAC increased significantly ($p<0.01$) by 40% during thiazide treatment to a similar degree in both groups of patients PRC increased slightly but significantly ($p<0.01$) by about 35% in both "responders" and non responders

Plasma potassium concentration decreased from 3 9 (3 4–4 1) to 3 3 mmol/l (2 5–4 0) ($p<0.01$) with no differences between the two groups of patients Plasma sodium concentration was 139 mmol/l (137–144) before treatment and did not change significantly 24-hour sodium excretion was 147 mmol (60–301) before and 148 mmol (73–316) during thiazide treatment There were no significant differences between the two groups of patients 24-hour potassium excretion was 63 mmol/l (30–144) before treatment and did not change during treatment

Angiotensin II blockade during thiazide treatment caused a significant decrease in MAP from *102 (91–116)* to 97 mmHg *(85–110)* ($p<0.01$) Every single "thiazide non responder had a clear-cut decrease in MAP (Fig 3) whereas 7 out of 11 thiazide responders did not show any fall in BP and 4 exhibited only a borderline transitory decline in MAP during angiotensin II blockade Presaralasin Map on thiazide was significantly higher in thiazide non responders than in responders' in concordance with our definition However during saralasin infusion MAP no longer differed significantly between the two patient groups (Table IV)

The agonistic effect of saralasin on adrenal receptors decreased or disappeared in the thiazide treated state but a consistent fall in PAC to values below preinfusion level was not seen In the thiazide responders" PAC increased by 33% ($p<0.05$) during saralasin infusion whereas the changes in thiazide non responders were insignificant PRC increased about 100% ($p<0.01$) during angiotensin

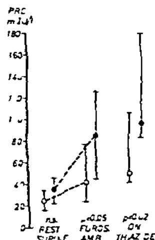


Fig 1 PRC at rest supine after furosemide + ambulation and during thiazide treatment in responders (O) and non responders (●) to thiazide treatment Note median value and interquartile range (central 50% range)

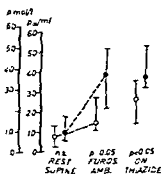


Fig 2 PA II at rest supine after furosemide + ambulation and during thiazide treatment in responders (O) and non responders (●) to thiazide treatment Note median value and interquartile range (central 50% range)

Table IV *Changes in MAP and PRC on saralasin infusion during thiazide treatment (median values with range in parentheses)*

	Presaralasin	On saralasin	Changes across saralasin (p)
MAP (mmHg)			
Thiazide responders	95 (91–113)	94 (85–110)	n s
Thiazide non responders	108 (93–116)	100 (86–106)	<0 01
	p<0 05	n s	
PRC (mIU/l)			
Thiazide responders	49 (35–142)	49 (34–[illegible])	n s
Thiazide non responders	96 (71–262)	198 (79–6[illegible])	<0 01
	p<0 02	p<0 01	

II blockade in the thiazide treated state in thiazide non responders but did not change significantly in thiazide responders (Table IV)

There was a close inverse correlation ($r=-0\ 70$ $p<0\ 001$) between preinfusion level of PA II and changes in MAP during saralasin infusion the fall in MAP being more pronounced when preinfusion PA II was high (Fig 4) On thiazide treatment a close correlation was found between preinfusion PA II and changes in PRC during saralasin infusion ($r=0\ 79$ $p<0\ 01$) the increase in PRC being largest with the highest PA II values

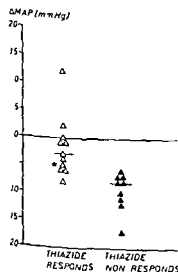


Fig 3 Changes in MAP on saralasin infusion during thiazide treatment ★=4 thiazide responders with a MAP fall of ≥5 mmHg who showed only a transitory decline ("borderline response") All thiazide non responders" exhibited a sustained decrease in MAP

At an infusion rate of 5 4 nmol/kg /min plasma saralasin concentration was 199 nmol/l (112–286) in the first and 188 nmol/l (107–381) in the second test Thus in molar terms the plasma concentration of the analogue was about 5000–20000 times higher than the plasma concentration of the endogenous octapeptide There were no significant differences between the plasma saralasin concentrations in the first and the second test

DISCUSSION

The present investigation deals with 40-year-old individuals with essential hypertension identified during a survey of a population born in 1936 In this group of patients PRC PA II and PAC at rest did not differ from reference values in 40-year-old normotensive controls selected from the same population Values after acute stimulation were also

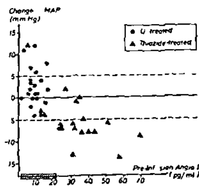


Fig 4 Change in MAP on saralasin infusion in relation to preinfusion angiotensin II concentration (pg/ml~pmol/l)

nearly identical Thus with this frame of reference the present homogeneous group of patients with mild essential hypertension showed no clear-cut division into renin subgroups In accordance with the proposal that low renin hypertension reflect a stage in the development of hypertension rather than a distinct entity (3) a classification into renin or angiotensin subgroups may well be difficult when age duration and severity of hypertension are identical

Normal values of PRC and PA II per se do not exclude the implication of RAS in the maintenance of hypertension However in the untreated sodium replete state angiotensin II blockade by means of saralasin caused a significant decrease in MAP in only two out of 25 patients and in the group as a whole MAP actually increased Similarly an agonistic effect of saralasin on adrenal receptors was discovered Thus in mild untreated essential hypertension angiotensin II has no decisive role in the maintenance of high BP as far as the sodium replete state is concerned This is in accordance with the results of other investigations in patients with essential hypertension Similarly in the state of a mild sodium depletion induced by furosemide or low salt diet before the infusion of saralasin no fall in BP was found in patients with low or normal renin hypertension (1 5 13 25) On the other hand a decrease in MAP was found during saralasin infusion after acute sodium depletion in patients with so-called high renin essential hypertension (13 25) In another group of patients with essential high renin hypertension a fall in BP was found during normal sodium balance when saralasin was infused with the patients in the sitting position (5 6) It is important to realize that saralasin has weak angiotensin II agonistic properties (1 5 16) which might lead to an underestimation of angiotensin II dependency particularly in normal renin hypertension (6 7) Blockade of the generation of angiotensin II by means of converting enzyme inhibitor (CEI) infused in the sitting position has been reported to induce a 10% fall in BP in patients with normal renin essential hypertension in the sodium replete state (6) Provided that an effect of CEI via inhibition of bradykinin metabolism can be ruled out the latter results point to the RAS as an important homeostatic mechanism in the maintenance of high BP even during normal sodium balance However it is possible that some part of the BP fall during CEI infusion is due to accumulation of bradykinin In fact a three fold increase in bradykinin concentration during CEI infusion has been reported in patients with hypertension (33)

The increase in PAC during saralasin infusion was very pronounced 126% This is significantly higher ($p<0.01$) than the increase of 72% found in normotensive subjects under the same conditions (16) In 2/3 of the hypertensive subjects the individual increase in PAC was above our normal range This marked agonistic response might well reflect the enhanced adrenal responsiveness to angiotensin II in patients with essential hypertension as reported by Kisch et al (21)

Based upon a positive correlation between age and plasma catecholamine concentration some investigators have claimed that the finding of elevated plasma catecholamine levels in patients with essential hypertension could be explained by age differences between the hypertensive patients and the normotensive controls (22 26) However this point has been refuted by other investigators (4 8) In the present group of hypertensive patients neither resting nor stimulated PNC differed from the reference values of the normotensive controls

In accordance with earlier investigations PV was slightly reduced in the present patients whereas total Na_E was normal (11 17 24)

An analysis of the individual responses to hydrochlorothiazide treatment revealed that the group of hypertensives could be divided into 11 "thiazide responders" and 10 "non responders" It is generally accepted that the antihypertensive effect of thiazides is related to their natriuretic properties (23 24) It has often been maintained that this effect depends almost entirely on volume depletion (23 31) This is especially true when thiazides are used in combination with other antihypertensive agents (11 18 24) During treatment with agents that interfere with sympathetic nerve activity BP becomes a direct function of intravascular volume and good BP control is dependent on the maintenance of a reduced intravascular volume (11) In some investigations a more marked volume reduction has been reported in low renin hypertension compared to normal or high renin hypertension when a thiazide is used alone (10) but in o[illegible] udies equal reductions in PV Na_E or ext[illegible] d [illegible] ve been found in "respon[illegible] on to the treatment (2 15 [illegible] ered that in r[illegible] fluid compar[illegible]

es in body weight (10 32 33) although it has been repeatedly shown that changing body weight across long term antihypertensive treatment is a very crude measure of changes in fluid compartments (15 18 24) Therefore direct measurements of fluid compartments are necessary Although the natriuretic effect is of importance for the antihypertensive action of thiazides this did not determine the antihypertensive response in the present investigation since the absolute values as well as the changes in PV and Na_E were similar in thiazide responders and non responders

The responsiveness of the RAS proved to be the determinant for the quantitative BP response to thiazide treatment This conclusion is supported by the much higher postthiazide PRC and PA II values in thiazide non responders than in responders The importance of RAS emerges even more convincingly from the outcome of the saralasin infusions in the thiazide treated patients showing that every single thiazide non responder exhibited a clear-cut decrease in MAP whereas only 4 out of 11 responders showed a borderline decrease There was a close inverse correlation between the preinfusion level of PA II and changes in MAP during saralasin infusion i e the contribution of angiotensin II to the BP regulation was most pronounced when PA II was high Furthermore when angiotensin II mediated vasoconstriction during thiazide treatment was blocked by means of saralasin MAP no longer differed significantly between the two groups of patients Thus an unsatisfactory response to thiazide was in fact due to hyperreactivity of RAS To put it in another way it must be assumed that the effect of the treatment would have been more satisfactory if the activation of RAS had not taken place With this in mind it might well be advantageous to consider the effect of adding a β-blocker to the treatment of patients with unsatisfactory response to thiazide Our preliminary results concerning addition of a β-blocker are compatible with the proposal that renin suppression is part of the antihypertensive mechanisms exerted by β-blockers in patients with activated RAS (23 27 29)

Our results agree by and large with those of other investigations concerning angiotensin II blockade during thiazide treatment (1 29) although selection of patients and duration of treatment are not directly comparable

Recently Weber et al (31) claimed that the changes in aldosterone secretion as defined from 24 hour urinary excretion rates play a major role in determining the outcome of diuretic treatment They found that the basal aldosterone excretion before treatment was significantly lower in non responders to treatment with chlorthalidone (100 mg/day for 6 weeks) than in responders but that the increase in excretion was more pronounced in the non responders than responders This conclusion is not supported by the results of measurements of PAC in our limited number of patients before and during thiazide treatment since PAC and changes in PAC were similar in thiazide responders and non responders

Several counter regulatory mechanisms might be operative during thiazide treatment e g the sympathetic nervous system as suggested by others (2) Determination of PNC by means of a sensitive double isotope derivative technique is currently thought to be a useful index of sympathetic nerve activity (9 22 26) During treatment PNC increased slightly but to a similar degree in thiazide non responders and responders Consequently differences in sympathetic nerve activity as defined from PNC measurements did not explain differences in the BP response to thiazide treatment

Several criteria have been published for classifying patients with essential hypertension into renin subgroups (10 23) No matter which frame of reference is used a number of investigations have shown that patients with low renin hypertension respond more satisfactorily to thiazide treatment than patients with normal or high renin essential hypertension (10 23 30) Although with reference to the normotensive control group our patients could not be classified into low normal and high renin subgroups before treatment it is pertinent that within the group of hypertensives a sizeable fraction disclosed hyperresponsiveness of the RAS during chronic thiazide treatment Instead of a division into static renin subgroups e g by means of the renin–sodium index it may be at least as meaningful and pragmatic from a pharmacotherapeutic point of view to define inappropriate responsiveness of the RAS as an unsatisfactory response to thiazide in conjunction with significant angiotensin II dependence of the BP during treatment Such a definition relates to the unsuccessful effect of thiazide treatment in some patients and emphasizes the need for alternative medication But assessment of the responsiveness of RAS during

acute furosemide stimulation before treatment may also be of value when selecting therapy since PA II after furosemide as well as the absolute increase in PA II across acute stimulation was much higher in thiazide non responders than in thiazide re sponders Thus without reference to values in normotensive control subjects assessment of an giotensin II response to acute stimulation could to a reasonable extent predict the subsequent response to thiazide treatment

CONCLUSION

The functional significance of the RAS in mild es *sential hypertension emerges after thiazide treat* ment Thiazide induced stimulation of RAS counterbalanced the hypotensive effect of thiazide in some 40% of the treated patients Thus the responsiveness of the RAS determined the quantita tive BP response to thiazide treatment Saralasin is a reliable tool for the assessment of the possible participation of angiotensin II in the maintenance of high BP

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REFERENCES

1 Anderson G H Dalakos T G Elias A Tomycz N & Streeten D H P Diuretic therapy and re sponse of essential hypertension to saralasin Ann In tern Med 87 183 1977

2 Beretta Piccoli C Weidmann P De Chatel R Hirsch D & Reubi F C Beziehungen zwischen Blutdruck Blutvolumen und plasmarenin wahrend Diuretika Therapie bei essentieller Hypertonie Schweiz Med Wochenschr 107 104 1977

3 Birkenhager W H & Schalekamp M A D H Control mechanisms in essential hypertension p 95 Elsevier Scientific Publishing Co Amsterdam Oxford and New York 1976

4 Campese V Myers M R & De Quattro V Plas ma catecholamines and neurogenic hypertension N Engl J Med 297 53 1977

5 Case D B Wallace J M Keim H J Sealey J E & Laragh J H Usefulness and limitations of sarala sin a partial competitive agonist of angiotensin II for evaluating the renin and sodium factors in hyperten sive patients Am J Med 60 825 1976

6 Case D B Wallace J M Keim H J Weber M A Drayer J I M White R P Sealey J E & Laragh J H Estimating renin participation in hyper tension Superiority of converting enzyme inhibitor over saralasin Am J Med 61 790 1976

7 Case D B Wallace J M Keim H J Weber M A Sealey J E & Laragh J H Possible role of renin in hypertension as suggested by renin sodium profiling and inhibition of converting enzyme N Engl J Med 296 641 1977

8 De Champlain J & Cousineau D Lack of correla tion between age and circulating catecholamines in hypertensive patients N Engl J Med 297 672 1977

9 Christensen N J Plasma noradrenaline and adrena line in patients with thyrotoxicosis and myxoedema Clin Sci Mol Med 45 163 1973

10 Dunn M J & Tannen R L Low renin hyperten sion Kidney Int 5 317 1974

11 Dustan H P Tarazi R C & Bravo E L Depen dence of arterial pressure on intravascular volume in *treated hypertensive patients N Engl J Med 286 861* 1972

12 Gavras H Brunner H R Laragh J H Sealey J E Gavras I & Vukovich R A An angiotensin converting enzyme inhibitor to identify and treat vaso-constrictor and volume factors in hypertensive pa tients N Engl J Med 291 817 1974

13 Gavras H Ribeiro A B Gavras I & Brunner H R Reciprocal relation between renin dependency and sodium dependency in essential hypertension N Engl J Med 295 1278 1976

14 Giese J Jorgensen M Nielsen M D Lund J O & Munck O Plasma renin concentration measured by use of radioimmunoassay for angiotensin I Scand J Clin Lab Invest 26 355 1970

15 Hunyor S N Zweifler J Hansson L Schork M A & Ellis C Effect of high dose spironolactone and chlorthalidone in essential hypertension Relation to plasma renin activity and plasma volume Aust N Z J Med 5 17 1975

16 Ibsen H Kappelgaard A M Nielsen M D & Giese J The effect of angiotensin II blockade by saralasin (1 Sar 8-alaangiotensin II) in normal man Eur J Clin Pharmacol 14 171 1978

17 Ibsen H & Leth A Plasma volume and extracellu lar fluid volume in essential hypertension Acta Med Scand 194 93 1973

18 Ibsen H Rasmussen K Jensen H Æ & Leth A Changes in plasma volume and extracellular fluid vo-lume after addition of prazosin to propranolol treat ment in patients with hypertension Scand J Clin Lab Invest 38 425 1978

19 Kappelgaard A M Ibsen H Nielsen M D & Giese J Radioimmunoassay for saralasin by means of anti angiotensin II sera Clin Chim Acta 83 25 1978

20 Kappelgaard A M Nielsen M D & Giese J Measurement of angiotensin II in human plasma Technical modifications and practical experience Clin Chim Acta 67 299 1976

21 Kisch E S Dluhy R G & Williams G H En hanced aldosterone response to angiotensin II in human hypertension Circ Res 38 502 1976

22 Lake C R Ziegler M G Coleman M D & Ko-pin I J Age adjusted plasma norepinephrine levels

are similar in normotensive and hypertensive subjects N Engl J Med 296 208 1977

23 Laragh J H Modern system for treating high blood pressure based on renin profiling and vasoconstriction volume analysis *A primary role for beta blocking* drugs such as propranolol Am J Med 61 797 1976

24 Leth A Body fluid compartments in essential hypertension Thesis Copenhagen 1976

25 Mayes D Furuyama S Kem D C & Nugent C A A radioimmunoassay for plasma aldosterone J Clin Endocrinol 30 682 1970

26 Pedersen E B & Christensen N J Catecholamines in plasma and urine in patients with essential hypertension determined by double isotope derivative techniques Acta Med Scand 198 373 1975

27 Pettinger W A & Mitchell H C Renin release saralasin and the vasodilator beta blocker drug interaction *in man* N Engl J *Med* 292 1214 1975

28 Streeten D H P Anderson G H & Dalakos T G Angiotensin blockade Its clinical significance Am J Med 60 817 1976

29 Vaughan E D Carey R M Peach M J Ackerly J A & Ayers C R The renin *response to diuretic* therapy A limitation of antihypertensive potential Circ Res 42 376 1978

30 *Vaughan* E D *Laragh J H Gavras I Buhler F* R Gavras H Brunner H R & Baer L Volume factor in low and normal renin essential hypertension Am J Cardiol 32 523 1973

31 Weber M A Drayer J I M Rev A & Laragh J H Disparate patterns of aldosterone response during diuretic treatment of hypertension Ann Intern Med 87 558 1977

32 Weber M A Lopez-Ovejero J A Drayer J I Case D B & Laragh J H Renin reactivity as a determinant of responsiveness to antihypertensive treatment Arch Intern Med 137 284 1977

33 Williams G H & Hollenberg N K Accentuated vascular and endocrine response to SQ 20881 in hypertension N Engl J Med 297 184 1977

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Angiotensin II in Primary Hypertension, Relationship to Plasma Renin Activity, Aldosterone and Urinary Electrolytes

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ABSTRACT Plasma concentrations of angiotensin II (A II) were studied in 36 patients with primary (essential) hypertension and 15 normotensive control subjects during basal (1 h supine rest), upright and frusemide stimulated (80 mg orally) conditions Plasma renin activity (PRA) and plasma aldosterone (PA) were determined on the same occasions A II was then correlated statistically to PRA PA and 24 hour urinary excretions of aldosterone (Aldo U), sodium and potassium and to the blood pressure (BP) levels The A II values in the hypertensive patients were not statistically significantly different from those in the normotensive subjects A close relationship was found between the A II values and the corresponding PRA values in the hypertensive patients ($r=0\ 65–0\ 76$, $p<0\ 001$ for all) Correlations between A II and PA, and A II and Aldo-U were not consistently significant No correlation was found between A II and BP or between A II and 24 hour urinary electrolytes The findings point to an intact function between PRA and A II but a disturbed A II–aldosterone interrelation in primary hypertension

Key words primary hypertension renin angiotensin II aldosterone relationships

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The renin–angiotensin–aldosterone (RAA) system is regarded as an interrelated functional unit and during normal conditions these hormones are in equilibrium (22) With the widespread use of radioimmunoassays detailed knowledge has been obtained about both plasma renin activity (PRA) and aldosterone secretion as participants in various hypertensive disorders (15 16 17 25 32) However the most important factor in the RAA system may be angiotensin II (A II) since it is the most potent naturally occurring vasoconstrictor substance in the body Attempts to measure A II directly have yielded conflicting results even though radioimmunoassay for A II has increased the sensitivity and specificity of such measurements There is general agreement that a rise in A II follows the administration of natriuretic agents (14 23) but the effect of dietary sodium restriction is disputed (3 9 19 21 31)

We studied A II during both basal and stimulated conditions (upright posture and frusemide 80 mg orally) and its relationship to the concomitant determinations of PRA and aldosterone in a selected population of hypertensive patients One aim was also to clarify if possible differences in the RAA system between the hypertensive and normotensive states and the relationship if any between A II and blood pressure (BP)

SUBJECTS

Thirty six hypertensive patients 20 men and 16 women were investigated at the Out Patient Department of Internal Medicine and the Clinical Research Center University Hospital Linkoping Sweden They ranged in age from 27 to 66 years (mean 47 6) All were judged to have primary hypertension and according to the WHO criteria all belonged to the classes I or II None had complicating diseases

The criterion for entering the study was a resting supine BP of >140/90 mmHg for patients up to 40 years of age and of ≥160/100 mmHg for older patients measured on at least two occasions No drugs including contraceptive pills were allowed All were untreated or had discontinued their antihypertensive therapy for at least four weeks before entering the study

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Abbreviations A I = angiotensin I A II = angiotensin II PRA = plasma renin activity PA = plasma aldosterone Aldo-U = urinary aldosterone RAA = renin-angiotensin-aldosterone BP = blood pressure

Blood samples were drawn at 8–10 a m For women with menstrual cycles the studies were performed in the follicular phase (days 5–10) Known causes of secondary hypertension were excluded by history physical examination and by normal findings of serum and urinary electrolytes serum creatinine 24-hour urinary vanillylmandelic acid urinary excretion of adrenaline and noradrenaline or methoxycatecholamines

Fifteen healthy normotensive volunteers 11 men and four women aged 24–70 years (mean 43 7) served as control subjects They were investigated identically regarding BP measurements and the procedure of the PRA–frusemide test (see below) All were normal on physical examination and biochemical testing and all denied intake of any medication including contraceptive pills or oestrogens

METHODS

BP measurements were performed in a standardized fashion by the same highly trained nurse (26) The BPs were measured in connection with the PRA–frusemide test supine after one hour s rest in the morning and sitting after 3–4 hours of ambulation (see below)

The PRA–frusemide test was performed as described in detail elsewhere (11) In brief measurements of PRA A II and plasma aldosterone (PA) were performed after one hour s rest in the morning after 3–4 hours of ambulation and 3–4 hours after oral administration of 80 mg frusemide Urinary aldosterone (Aldo-U) and urinary sodium and potassium excretions were determined from urine collected during the 24 hours preceding the PRA–frusemide test

Blood for PRA A II and PA determinations was collected in ice-cold EDTA vacutainer tubes and placed in ice Plasma was separated within 20 min by centrifugation at +4°C for 15 min and then stored at −20°C until assayed

PRA was determined by a radioimmunoassay method described previously (8) Results are expressed as angiotensin I (A I) generated per litre per hour (pkat l⁻¹) Details of the PRA method as used in our laboratory regarding sensitivity and reproducibility as well as reference ranges have been published elsewhere (11)

Radioimmunoassay for A II was performed with minor modifications according to Kosunen (13) The method is relatively simple and permits determinations of A II in 0 5–1 ml samples of plasma Interfering proteins are eliminated by ion exchange chromatography on Dowex 50W X2 The A II rabbit antiserum used in this assay crossreacts in per cent with A I 7 1 the tetradecapeptide 4 6 the heptapeptide (2–8) (A III) 58 and the hexapeptide (3–8) <0 03 Asp -Ileu⁵-Angiotensin II (A 70/302) from the Medical Research Council London England was used as standard

All measurements were performed in duplicate The precision for the A II method in our laboratory is coefficient of variation for intra assay ($n=57$) 9% for samples with a mean A II concentration of 60 pmol l⁻¹ for interassay ($n=11$) 17–30% for three control samples within the range of 28–65 pmol l⁻¹ The reference ranges ($\bar{X}\pm$S D) given by Kosunen are for resting conditions 29 9±10 3 pmol l⁻¹ and after 2 hours of ambulation 40 7±12 3 pmol l⁻¹ The accuracy of the A II method was tested by adding increasing amounts (25–150 pmol) of unlabelled A II to plasma. The correlation between added A II and the A II found by radioimmunoassay was excellent ($r=0.98$ $n=16$) With the routine method used the mean recovery of A II through the column was 79.5% (range 61–100) in 101 plasma samples The A II method correlated well with our PRA method when 95 random plasma samples over a wide PRA range were analyzed ($r=0.74$ $p<0.001$)

Plasma and urinary aldosterone were measured by specific radioimmunoassay methods without chromatography (4 18) as described in detail elsewhere (28) Reference values for this method in our laboratory have been published (28)

Statistical methods

Standard statistical methods were used to determine mean values S D and the correlation and partial correlation coefficients Student s t-test was used for comparison between the mean values of the groups The calculations were performed at the Computer Center in Lund using the SPSS package (20) Statistical analyses were carried out using an Olivetti Programma 101 P levels between 0 05 and 0 001 were considered statistically significant

RESULTS

Angiotensin II

The results of the A II determinations during supine rest upright posture and after frusemide stimulation for the hypertensive patients are shown in Fig 1 and for the normotensive controls in Fig 2 The mean A II level rose about 45% when changing from supine to upright position and about 85% from supine to frusemide stimulated values for both the hypertensive and the normotensive population Strong correlations existed between basal supine A II and both upright ($r=0.88$ $p<0.001$) and stimulated A II ($r=0.76$ $p<0.001$) for the individual patient despite wide ranges for all these determinations Comparison of the hypertensive patients with the normotensive controls revealed no significant differences between their basal upright or stimulated A II levels

PRA PA and Aldo-U

The mean ± S D values for PRA and PA at the same defined conditions as for A II are shown in Figs 3 and 4 Comparison of the upright and the frusemide stimulated mean levels of A II and PRA demonstrated that these two parameters increased nearly identically from the basal levels (Figs 1 and 3) The PA response to the stimulative manoeuvres

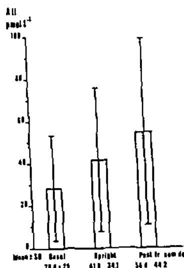


Fig 1 Mean plasma concentration of A II in the hypertensive patients in connection with the PRA–frusemide test

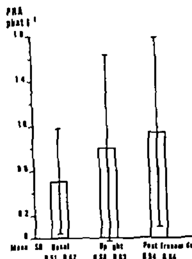


Fig 3 Mean PRA in 36 hypertensive patients Samples were drawn after 1h rest in the sitting position after 3–4 h ambulation and after stimulation with 80 mg frusemide orally

differed from this pattern Mean PA rose 130% with change from supine to upright posture with hardly any further increase after frusemide stimulation (Fig 4) The 24-hour Aldo-U was 27±16 nmol for the entire hypertensive population All values fell within the reference ranges for our laboratory (28)

BP and pulse rates

The BPs of the patients after 1 hour s rest were 173±19 mmHg (mean±S D) systolic and 111±7 mmHg diastolic The corresponding BPs in the sitting position after 3–4 hour s ambulation were systolic 170±17 and diastolic 110±7 The mean resting and upright pulse rates were 74±11 and 89±11 BPM respectively

Urinary electrolytes serum creatinine, endogenous creatinine clearance and body weight

Mean±S D values for the following parameters for the hypertensive subjects were 24 hour urinary sodium and potassium excretion 161±73 and 68±28 mmol respectively serum creatinine 84±17 μmol

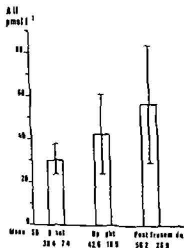


Fig 2 Mean plasma concentration of A II in 15 normotensive subjects in connection with the PRA–frusemide test

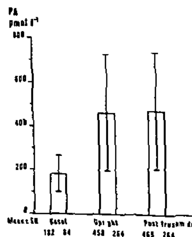


Fig 4 Mean PA levels in the hypertensive patients after 1h rest in the sitting position after 3–4 h ambulation and after frusemide stimulation

Table I *Correlations between PRA A II PA and Aldo U in connection with PRA–frusemide test in 36 hypertensive patients*

	r	p
Basal PRA vs basal A II	0 65	<0 001
Upright PRA vs upright A II	0 76	<0 001
Stimulated PRA vs stimulated A II	0 71	<0 001
Basal A II vs basal PA	No correlation	
Upright A II vs upright PA	0 48	<0 01
Stimulated A II vs stimulated PA	0 44	<0 05
Basal A II vs Aldo U	No correlation	
Upright A II vs Aldo-U	No correlation	
Stimulated A II vs Aldo U	0 35	<0 05

l^{-1} endogenous creatinine clearance 116±34 ml/min and b wt 75±13 kg These values fell within the reference ranges for our laboratory

Relations between A II and other parameters

A II versus PRA PA and Aldo-U Significant relationships existed between both basal upright and stimulated A II and corresponding PRA values (r= 0 65 r=0 76 r=0 71 respectively p<0 001 for all) The correlations between A II and PA were not so obvious No correlation existed between basal A II and basal PA but such was found between both upright and stimulated A II and the corresponding PAs (r=0 48 p<0 01 and r=0 44 p<0 05) The relations between A II and Aldo-U were even weaker Only between stimulated A II and Aldo-U did we find a significant correlation (r=0 35 p<0 05) (Table I)

A II versus urinary electrolytes serum creatinine endogenous creatinine clearance and body weight No statistically significant correlations at all were found between either of the three A II values and the concomitant 24 hour urinary sodium and potassium excretions serum creatinine endogenous creatinine clearance or body weight

A II versus BPs and pulse rates A II for the hypertensive patients did not correlate statistically with the systolic and diastolic supine and sitting BPs or the resting and sitting pulse rates measured in connection with the PRA-frusemide test

DISCUSSION

A II may be the most relevant hormone in the RAA system regarding BP regulation as it is the main active component of the system in the circulation However there have been considerable methodological difficulties in obtaining reliable measurements of A II (1 7) The radioimmunoassay used in this study seems to be reliable The results of the A II determinations in our controls agreed fairly well with what some authors have found (5 13 29) but were somewhat higher than those reported by others (2 6 31) These differences may be explained by heterogeneous patient groups with varying salt intakes as well as methodological differences

We found strongly positive correlations between basal upright and frusemide stimulated A II values and the corresponding PRA values in the hypertensive subjects Thus we noticed proportional changes in *A II* as well as in *PRA* after upright posture and frusemide stimulation which findings are in agreement with those of Kuppers et al (14) but in contrast to other published results (5 10 30) The relationship between A II and aldosterone was weaker We have previously reported highly significant correlations between PRA and PA in a normotensive population (28) but in the present group of hypertensive patients the PRA and A II values versus the PA and Aldo U values were not consistently correlated (Table I) Only the frusemide stimulated A II correlated significantly with 24 hour urinary aldosterone excretion (p<0 05) This finding indicates that the connection between PRA and A II is intact in primary hypertension and there is no inverse relationship between PRA and A II as proposed by Walker et al (30) Many of our patients had very low and unstimulated PRA values but also low and unstimulated A II levels Thus our results point towards a disturbed relation between A II and aldosterone in primary hypertension compared with the normal state It seems unlikely that A II was the main factor responsible for the changes in PA from basal to upright and stimulated levels (Figs 1 and 4) and there were clear discrepancies between these changes in A II and the corresponding PA values Volume and electrolyte changes may explain the PA responsiveness in connection with the PRA–frusemide test

We have previously observed that no relationship exists between PRA and 24 hour urinary sodium and potassium excretions either in normotensive or hypertensive subjects on unrestricted diets (12) In this study no correlations were found between basal upright or stimulated A II and the urinary

sodium or potassium excretions This contrasts with what other authors have found for normotensive subjects (9 24) However we obtained a positive significant relationship between Also-U and 24 hour urinary potassium excretion This relation between Aldo-U and urinary potassium has also been noticed in the previously mentioned study of hypertensive patients but could not be found in the normotensive control subjects (28) These findings also support the concept of a disturbed relation between A II and aldosterone and/or abnormal regulation of aldosterone in primary hypertension Whether this disturbance of the RAA system is a primary pathogenetic factor or a secondary phenomenon is impossible to establish from this study

Comparison of the A II levels with the BPs revealed no significant relationship This differs from what Walker et al (30) have published and our results contradict the suggestion that the elevated BP in primary hypertension is maintained by endogenous A II

In conclusion the present study has shown that basal and stimulated A II levels in patients with primary hypertension did not differ from those in the normotensive controls Further a close relationship exists between A II values and the corresponding PRA values but the correlation to the aldosterone excretion is not obvious indicating a disturbed relation between A II and aldosterone even in patients with primary hypertension

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REFERENCES

1 Barrett J D Eggena P & Sambhi M P Extraction and measurement of circulating angiotensin I and II Clin Chem 23 464 1977
2 Beevers D G Morton J J Nelson C S Padfield P L Titterington M & Tree M Angiotensin II in essential hypertension Br Med J 1 415 1977
3 Brown J J Lever A F Morton J J Fraser R Love D R & Robertsson J I S Raised plasma angiotensin II and aldosterone during dietary sodium restriction in man Lancet 2 1106 1972
4 Brown R D Swander A & McKenzie J K Urine aldosterone radioimmunoassay validation of a method without chromatography J Clin Endocrinol 42 894 1976
5 Catt K J Cain M D Zimmet P Z & Cran E Blood angiotensin II levels of normal and hypertensive subjects Br Med J 1 819 1969
6 Dusterdieck G & McElwee G Estimation of angiotensin II concentration in human plasma by radioimmunoassay Some applications to physiological and clinical states Eur J Clin Invest 2 32 1971
7 Fyhrquist F Radioimmunoassay of plasma angiotensin II in patients with hypertension in renal transplant recipients and in anephric patients Soc Sci Fenn Comment Biol (Suppl) 39 1 1971
8 Fyhrquist F Soveri P Puutula L & Stenman U H Radioimmunoassay of plasma renin activity Clin Chem 22 250 1976
9 Gocke D J Gerten J Sherwood L M & Laragh J H Physiological and pathological variations of plasma angiotensin II in man Circ Res (Suppl) 1 131 1969
10 Horvath J S Moore M A Russell R P & Walker W G Abnormalities of plasma renin activity (PRA) and venous angiotensin II (A II) in essential hypertension Aust NZ J Med 4 422 1974
11 Karlberg B E & Tolagen K Relationships between blood pressure age plasma renin activity and electrolyte excretion in normotensive subjects Scand J Clin Lab Invest 37 521 1977
12 — Age blood pressure renin and urinary electrolyte in primary hypertension and in the normotensive state Scand J Clin Lab Invest 38 319 1978
13 Kosunen K J A simple method for measurement of angiotensin II in plasma Scand J Clin Lab Invest 36 467 1976
14 Kuppers H Wiesen K & Schnurr E Determination of plasma angiotensin II concentration Rehability of a practical method for clinical purposes R A A system during furosemide administration Clin Chim Acta 71 469 1976
15 Laragh J H Sealey J E Buhler F R Vaughan F D Brunner H R Gavras H & Baer L The renin axis and vasoconstriction volume analysis for understanding and treating renovascular and renal hypertension Am J Med 58 4 1975
16 London G M Safar M E Weiss Y A Corvol P L Menard J E Simon A C & Milliez P L Relationship of plasma renin activity and aldosterone levels with hemodynamic functions in essential hypertension Arch Intern Med 137 1042 1977
17 Luetscher J A Ganguly A Melada G A & Dowdy A J Abnormal regulation of aldosterone in hypertension Adv Intern Med 20 183 1975
18 McKenzie J K & Clements J A Simplified radioimmunoassay for serum aldosterone utilizing increased antibody specificity J Clin Endocrinol 38 622 1974
19 Mendelsohn F A O Johnston C L Doyle A E Scoggins B A Denton D A & Coghlan J P Renin angiotensin II and adrenal corticosteroid relationships during sodium deprivation and angiotensin infusion in normotensive and hypertensive man Circ Res 31 728 1972

20 Nie N H Bent D H & Hull C H Statistical package for the social science McGraw Hill New York 1970
21 Nielsen I & Moller I Simultaneous determination of renin activity and angiotensin concentration levels in human plasma Acta Med Scand 182 263 1967
22 Oparil S & Haber E The renin angiotensin system N Engl J Med 291 389 446 1974
23 Page L B Haber E Kimura A Y & Purnode A Studies with the radioimmunoassay for angiotensin II and its application to measurement of renin activity J Clin Endocrinol 29 200 1969
24 Roser E A Brown J J Cumming A M M Fraser R Semple P F Lever A F Morton J J Robertson A S Robertson J I S & Tree M Is the sodium index a useful way of expressing clinical plasma renin angiotensin and aldosterone values? Clin Endocrinol 8 141 1978
25 Swales J D Low renin hypertension nephrosclerosis? Lancet 1 75 1976
26 Thulin T Andersson G & Schersten B Measurement of blood pressure a routine method in need of standardization Postgrad Med J 51 390 1975
27 Tolagen K Aldosterone in primary hypertension Relationship to plasma renin activity and urinary electrolytes and a comparison with normotensive subjects Scand J Clin Lab Invest 38 487 1978
28 Tolagen K & Karlberg B E Plasma and urinary aldosterone and their interrelations with blood pressure plasma renin activity and urinary electrolytes in normotensive subjects Scand J Clin Lab Invest 38 241 1978
29 Tuck M L Dluhy R S & Williams G H Sequential responses of the renin–angiotensin–aldosterone axis to acute postural change effect of dietary sodium J Lab Clin Med 86 754 1975
30 Walker W G Horvath J S Moore M A Whelton P & Pattersson Russell R Relation between plasma renin activity angiotensin and aldosterone and blood pressure in mild untreated hypertension Circ Res 38 470 1976
31 Walker W G Moore M A Horvath J S & Whelton P K Arterial and venous angiotensin II in normal subjects Circ Res 38 477 1976
32 Wisenbaugh P E Garst J B Hull C Freedman R J Matthews D N & Hadady M Renin aldosterone sodium and hypertension Am J Med 52 175 1972

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Heart and Kidney Involvement and Prognosis in Hypertension

A Study Concerning Referred Hypertensive Patients and Hypertensive Patients Found by Blood Pressure Screening

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ABSTRACT Severity of hypertension, frequency of secondary hypertension and prognosis have been compared in two groups of hypertensive men The first group ($n=686$) was taken from a blood pressure screening of a total population sample The other group ($n=154$) consisted of hypertensive men referred to a hypertension clinic by physicians The mean age of the groups was the same, ($\bar{x}=52$ years, range 46–59 years) All went through the same investigations and were followed up and treated in a similar way at the hypertension clinic The referred men had more severe hypertension as shown by significantly more heart and kidney involvements They also had a higher incidence of myocardial infarction, implying a poorer prognosis with regard to cardiovascular disease The analysis shows the importance of a detailed description of studied groups, not only in terms of blood pressure, age and sex, but also with respect to the frequency and degree of present and previous signs of heart and kidney involvement With such a description it is possible to compare results from different studies regarding pathophysiological mechanisms and the effect of treatment in hypertension

Key words essential hypertension heart function kidney function prognosis
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Authors have obtained different results when studying pathophysiological mechanisms leading to hypertension The same may be said about trials of the beneficial effects of hypertensive treatment This is probably partly due to the different selection of the studied groups with respect to age sex race and severity of hypertension Most reports on morbidity and mortality in hypertension have been based on hospitalized groups of hypertensives such as those presented by the Veterans Administration Cooperative Study Group (11) The vascular and renal abnormality in this study was highly prevalent 65% implying severe hypertensive disease These results cannot be generalized to large hypertensive populations found by community screening In another selected hypertensive population described by Brunner (3 4) the high renin subgroup had a higher diastolic blood pressure (DBP) a higher frequency of increased blood urea and more severe retinal changes than the low renin subgroup Thus the high renin subgroup seemed to have more advanced hypertensive disease on entry into the trial This rendered it more difficult to compare subsequent complications of hypertension in the two groups The finding in the two studies of a better prognosis for low renin hypertensives could not be verified in a subsequent study where patients with less severe hypertension were included (1)

These are but examples which could be multiplied They show how the selection of material and hence the severity of the hypertensive disease might influence the results and/or their interpretation

The aim of our study is to compare blood pressure (BP) severity of the hypertensive disease the prevalence of secondary hypertension and the prognosis in two groups of hypertensive men 1) obtained by BP screening of a total population sample 2) hypertensive men of the same age referred from a physician to the Hypertension Clinic

Abbreviations BP = blood pressure DBP = diastolic BP SBP = systolic BP MI = myocardial infarction

Table I *Diastolic blood pressure at initial visit at the Hypertension Clinic*

DBP (mmHg)	Referred group (*n* 154)			Population based group (*n* 686)		
	n	%		*n*	%	
<95	12	7.8		114	16.6	
95–104	33	1 4		313	45.6	
105–114	53	34.4	70.8	109	15.9	37.8
>115	56	36.4		150	?1 9	

$p<0.0005$

STUDY POPULATIONS

The population based group of hypertensives was recruited from a multifactorial primary preventive trial against myocardial infarction (MI) and stroke. This started in 1970 (13). In this trial a randomly selected third (*n* 9996) of all men born 1915–19 2 and 19 4–1925 living in Goteborg were invited to a screening examination including BP measurement. 7455 (75%) attended. Those on current therapy and those having a BP above 175 mmHg systolic or above 115 mmHg diastolic on screening and on examination two weeks later formed the population based group (*n* 686) previously described by Berglund et al (2). The mean age was 51.5+2.3 years.

The group of selected hypertensives consisted of 154 men referred to our Outpatient Hypertension Clinic between 1970–1975. They were 46–59 years old ($\bar{X}$= 52.2+3.3) referred from general practitioners (21%), hospital outpatient clinics (0%), industrial health care (19%), internal medicine outpatient clinics (18%), private physicians (18%) and hospital wards (5 ε). The reasons for referral were obstacles in obtaining adequate BP reduction, suspicion of secondary hypertension or the fact that the referring doctor was unaccustomed to handle patients with hypertension. Thirty nine (?3 3%) men in the referred group and 75 (10 7%) in the population based group had been admitted to hospital and examined because of hypertension before their first visit to the Hypertension Clinic.

METHODS

At the screening examination of the population based group which took place between 4.30 and 7.00 p.m. BP was measured in the right arm with the patient in a sitting position after a short interview with a physician. A standard cuff 12 cm wide 6 cm long connected to a mercury manometer was used. Pressures were read to the nearest ? mmHg. DBP was read when the Korotkoff sounds disappeared. A second measurement was taken two weeks later on subjects with a systolic blood pressure (SBP) above 175 mmHg or a DBP above 115 mmHg at screening. This measurement was taken after the subject had been at rest in a sitting position for about five minutes.

At the Outpatient Hypertension Clinic all casual BP recordings were made after five minutes supine rest by specially trained nurses using the standard cuff connected to a mercury manometer. Three BPs were measured before the first visit to a physician. The third measurement has been used as the initial blood pressure. In patients with an extremely high BP the first recording has been used as treatment had to be started or changed directly at the first visit to the clinic.

The investigations included history taking and a complete physical examination with auscultation of the heart and lungs, palpation of peripheral pulses and examination of the ocular fundi. Laboratory tests were taken including measurement of serum electrolytes, creatinine, cholesterol, triglycerides, urine tests for albuminuria, urinary sediment and urine culture. Renal concentration capacity after 13 hours without liquid was determined according to the method of Hood et al (8). If the result was below 700 mOsm/kg H_2O a vasopressin tannate test was performed. Serum electrolytes and creatinine were determined using a Technicon AutoAnalyzer and albuminuria with a dip test (Albustix Ames). Isotope renography was performed in 287 men (41.8%) in the population based group and in 66 (4? 8%) in the referred group using the standard method and apparatus (Nucab Sweden). Intravenous pyelography using a standard method was performed in 52 men (7.6%) in the population based group and in 42 (27.3%) in the referred group. Renal aortography was carried out in 12 men (1.7%) in the population based group and in 17 men (11%) in the referred group. On the basis of the results of these investiga

Table II *The frequency of secondary and primary hypertension and the frequencies of patients with primary hypertension in WHO stages I–III*

	Referred group (*n* 154)		Population based group (*n* 686)		$p<$
	n	%	*n*	%	
Secondary hypertension	12	7.8	40	5.8	n.s.
Primary hypertension	142	9 2	646	94 ?	n.s.
WHO I	29	0 4	56	39.6	0.0005
WHO II	57	40.1	48	38.4	n.s.
WHO III	55	38.7	135	0 9	0.0005
Missing data	1	0.7	7	1.1	

Table III *The frequency of previous diseases at the initial examination*

	Referred group (n=154)		Population based group (n=686)		
	n	%	n	%	p<
Myocardial infarction	7	4 5	19	2 8	n s
Angina pectoris	23	14 9	40	5 8	0 001
Heart decompensation	2	1 3	12	1 7	n s
Intermittent claudication	3	1 9	20	2 9	n s
Stroke	4	2 6	13	1 9	n s
Diabetes mellitus	5	3 2	27	3 9	n s
Gout	5	3 2	15	2 8	n s

tions and a thorough examination of the case notes pa tients with secondary hypertension were recognized Further details regarding the criteria for secondary hypertension have been described earlier (2) When the criteria for primary renal disease were not fulfilled serum creatinine values above 114 4 μmol/l and/or albuminuria on at least two separate occasions were regarded as signs of renal disease secondary to primary hypertension

Electrocardiograms were coded in accordance with the Minnesota code (10) As signs of left ventricular hypertrophy the combination of amplitude criteria 3 1 or 3 3 and S-T or T criteria 4 1-3 or 5 1-3 were used Left ventricular conduction defects i e left bundle branch block (7 1) and left anterior hemiblock (2 1) were also recorded For the WHO-classification chest films were analyzed by an experienced roentgenologist for signs of rounded or prominent left ventricle Heart size was calcu lated according to Jonsell (9) Heart enlargement was de fined as a relative volume of ≥500 ml/m² BSA A previous study of middle aged men in Goteborg showed that only 5% of normotensive men reach this level A relative vol ume of ≥550 ml/m BSA was supposed to indicate cardiac dilatation advanced enough to be regarded as evidence of organic damage attributable to the hypertensive disease and hence used for the classification into WHO stage III

Fatal and non fatal myocardial infarctions and strokes in our study were followed by registers (6 7) covering at least 90% of new cases Personnel looking after the register had no knowledge about the groups which the registered patients belonged to Each patient was followed for two years The definitions of MI and stroke were those sug gested by WHO although for MI a slightly modified ver sion a study made in Goteborg Sweden was used (6)

Angina pectoris and intermittent claudication were de fined according to Rose and Blackburn (10) Heart de compensation diabetes mellitus and gout were diagnosed by conventional methods

Treatment policy was not rigorously standardized dur ing the follow up but mostly treatment was started with a beta adrenoceptor blocking agent or a saluretic diuretic adding hydralazine to either drug or to the combination If normotension was not achieved with these drugs spironolactone bethanidine alpha methyl-dopa high dose furosemide or other drugs used in hypertension were added

Standard methods were used for the calculation of means and standard deviations The hypothesis of no dif ferences in means between the two groups was tested with Student s t test for unpaired observations The hypothesis of no differences in proportions was tested with the χ^2 test Only two-sided tests were used

RESULTS

Blood pressure

At the initial investigation at the Hypertension Clinic 68 8% (n–106) of the referred group and

Table IV *ECG changes associated with hypertension*

	Referred group (n=141)		Population based group (n=635)		
	n	%	n	%	p<
High QRS amplitude (3 1 or 3 3)	33	23 4	75	11 8	0 001
ST or T changes (4 1-3 or 5 1-3)	41	29 1	169	26 6	n s
High QRS amplitude+ST or T changes (3 1 or 3 3 4 1-3 or 5 1-3)	14	9 9	40	6 3	n s
Left ventricular conduction defects					
LAH (2 1)	5	3 5	29	4 6	n s
LBBB (7 1)	3	2 1	3	0 5	n s

Patients with primary hypertension with coded ECG

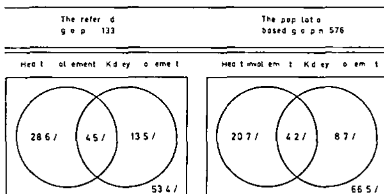


Fig 1 Frequency of heart involvement on X ray (≥500 ml/m² BSA) or ECG (3 1 or 3 3 and 4 1–3 or 5 1–3) and the frequency of kidney involvement (serum creatinine 114 4 μmol/l or two positive test for albuminuria) * Patients with primary hypertension and available data regarding heart and kidney involvement

32 1% (n=220) of the population based group were on antihypertensive treatment The initial BP was significantly higher (p<0 001) in the referred group (SBP 179±25 mmHg DBP 112±12 mmHg) than in the population based group (SBP 169±21 mmHg DBP 106±13 mmHg) in spite of more treated persons in the referred group The distributions of DBP (Table I) showed a significantly higher proportion of patients in the referred group with BP above 105 mmHg

Frequency of secondary hypertension

In the population based group the frequency of secondary hypertension was 5 8% (n=40) 3 5% being due to renoparenchymal disease In the referred group 7 8% (n=12) had secondary hypertension hus there was no significant difference in preva nce of secondary hypertension between the two groups (Table II)

WHO classification

When the patients were classified into three stages of hypertensive disease according to the norms drawn up by WHO (14) (Table II) we found a higher proportion (40%) of persons without evidence of organic changes in the cardiovascular system in the population based group than in the referred group (20%) Likewise there was a significantly higher proportion of WHO stage III in the referred group

Severity of the hypertensive disease

At the first examination at the Hypertension Clinic the frequency of previous MI heart decompensation intermittent claudication stroke and diabetes mellitus did not differ significantly between the two groups (Table III) The only significant difference between the groups was the prevalence of angina pectoris which was two and a half times higher in the referred group (p<0 001)

The frequency of hypertensive organ manifestations at the initial examination at the clinic is shown in Table IV and Figs 1 and 2 In these analyses all cases with secondary hypertension have been excluded

The mean value for relative heart volume was 467 ml/m² BSA in the referred group and 437 ml/m² BSA in the population based group The difference was statistically significant (p<0 05) Heart enlarge

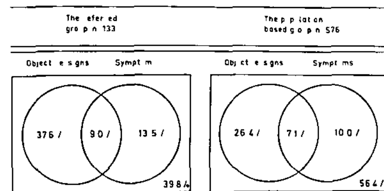


Fig 2 Frequency of reported symptoms and objective signs of cardiovascular disease * Patients with primary hypertension and no missing data

ment (heart volume ≥500 ml/m² BSA) was also significantly ($p<0.05$) more common in the referred group

Table IV shows the frequency of different ECG changes A higher proportion of high R waves was seen in the referred group whereas no differences were found between the two groups regarding ST T changes a combination of voltage changes and ST T changes or left ventricular conduction defects

Evidence of renal damage was found in a slightly higher proportion in the referred group (Fig 1) The difference between the groups in this respect however was not statistically significant

Fig 1 shows the frequency of heart and kidney involvement The total number of patients exhibiting either heart involvement or renal damage was significantly higher in the referred group ($p<0.01$) The overlapping between the two types of organ manifestations was the same in the two groups

There was a poor concordance between symptoms of cardiovascular disease (angina pectoris heart decompensation and intermittent claudication) and objective signs (heart enlargement on X ray and/or left ventricular hypertrophy on ECG and/or kidney damage) of cardiovascular disease (Fig 2) There was no difference between the groups in this respect

Morbidity and mortality in MI and stroke

The incidence of fatal and non fatal MI was significantly higher ($p<0.001$) in the referred group (12/154 7.8%) than in the population based group (11/686, 1.6%) during the two years of follow up The mortality from MI was 3.9% ($n=6$) in the referred group and 0.6% ($n=4$) in the population based group No significant differences were found regarding fatal and non fatal stroke Among the referred patients one (0.6%) had a non fatal stroke No one died from stroke The corresponding figures in the population based group were four (0.6%) and two (0.3%)

Other risk factors for coronary heart disease

51% were smokers in the referred group and 38% in the population based group Serum cholesterol was 6.3±1.1 mMol/l and triglycerides were 1.7±0.9 mMol/l in the referred group and 6.6±1.2 mMol/l and 1.7±0.9 mMol/l respectively in the population based group i e no significant differences Body weight was almost the same in the referred group (82.1±13.4 kg) and in the population based group (82.7±12.2 kg)

DISCUSSION

The two groups of hypertensives presented in this paper belonged to the same middle aged population in Goteborg The population based group can be considered representative for an unselected series of hypertensives where all stages of the disease have the same chance of being included On the other hand the referred group is most probably representative for hypertensive middle aged men treated at any hypertension clinic in similar populations If screening had not been performed some men whose hypertension was now discovered by this procedure might otherwise have entered the referred group having been detected by the industrial health care or the usual governmental primary care system

Both groups of hypertensives went through the same investigations and were examined and followed concurrently by the same physicians Therefore the results are not influenced by methodological differences

Our results showed that the patients in the referred group had a more severe form of hypertension than the men discovered by epidemiological methods The former had a higher BP and more frequent signs of heart and kidney involvement They also had a higher prevalence of angina pectoris and a higher incidence of MI indicating a poorer prognosis

Surprisingly enough the two groups did not differ significantly regarding definite signs of left ventricular hypertrophy on ECG This finding was rather uncommon in both groups 9.9% among the referred patients and 6.3% in the population based group The corresponding figure in the Veteran Administration Study was 16% (11) indicating that these patients had a more severe hypertension

It has previously been shown that electrocardiographic evidence of left ventricular hypertrophy does not necessarily imply that heart enlargement is to be seen on X ray In our two groups of hypertensive men about 50% of those who had definite left ventricular hypertrophy on ECG had larger hearts than average This implies that methods commonly used to diagnose myocardial hypertrophy have a low sensitivity and probably also a low specificity In previous studies our

group has found orthogonal ECG to be a better discriminator between normo- and hypertensives than both conventional ECG and X ray (12)

The poor concordance between objective signs of organic changes in the cardiovascular system and clinical symptoms of cardiovascular disease might partly be explained by the lack of sensitivity of the diagnostic methods used However it seems that the manifestations of sustained high BP differ from person to person Our results indicate that both history taking and laboratory investigations of various types are necessary to identify hypertensive patients with an increased risk

The prevalence of secondary hypertension was rather unexpectedly not significantly more common in the referred group This might be explained by the fact that when suspicion of a specific renal or endocrinological disorder arose the patients were probably referred directly to the nephrological or to the endocrinological department at the hospital

Many clinical reports on hypertensive disease from different centres deal with pathophysiological mechanisms and the effect of treatment It is important that results from different centres can be compared with each other To make such comparisons possible the severity of hypertensive disease in the studied groups must be known as both pathophysiological mechanisms and response to treatment may vary with the severity of hypertension Careful descriptions of the materials are there re needed not only in terms of BP age and sex ut also with respect to the frequency and degree of present and previous signs of heart and kidney involvement Our analysis shows that it is important to report in detail how the diagnosis of organ manifestations is made and that both qualitative and quantitative derangements from normal will be considered

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REFERENCES

1 Amery S Stroobant R & Fagard R Prognosis in low renin hypertension N Engl J Med 228 267 1973
2 Berglund G Andersson O & Wilhelmsen L Prevalence of primary and secondary hypertension Studies in a random population sample Br Med J 2 554 1976
3 Brunner H R Laragh J H Baer L Newton M A Goodwin F T Krakoff L R Bard R H & Buhler F R Essential hypertension Renin and aldosterone heart attack and stroke N Engl J Med 286 9 1972
4 Brunner H R Sealey J E & Laragh J H Renin subgroups in essential hypertension Further analysis of their patho-physiological and epidemiological characteristics Circ Res (Suppl) 1 1973
5 Doyle A E Jerums J Johnston C I & Louis W J Plasma renin levels and vascular complications in hypertension Br Med J 2 206 1973
6 Elmfeldt D Wilhelmsen L Tibblin G Vedin J A Wilhelmsson C & Bengtsson C J Registration of myocardial infarction in the city of Goteborg Sweden J Chronic Dis 28 173 1975
7 Harmsen P & Tibblin G A stroke register in Goteborg Sweden Acta Med Scand 191 463 1972
8 Hood B Carlsson M Falkheden T & Bengtsson U Ett förkortat torstprov och dess tillförlitlighet Nord Med 74 1111 1965
9 Jonsell S A method for determination of the heart size by teleroentgenography (a heart volume index) Acta Radiol 20 325 1939
10 Rose G A & Blackburn H Cardiovascular survey methods WHO Monogr Ser 56 1968
11 Veterans Administration Cooperative Study Group on Antihypertensive Agents Effect of treatment on morbidity in hypertension Circulation 45 991 1972
12 Wikstrand J Berglund G & Wilhelmsen L Non invasive assessment of cardiac function in normo and hypertensive 50-year-old males Eur J Cardiol 4/3 402 1976
13 Wilhelmsen L Tibblin G & Werko L A primary preventive study in Gothenburg Sweden Prev Med 1 153 1972
14 WHO Tech Rep Ser 231 1962

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A Follow-up Study of Hypertensive Patients after Operative Treatment of Unilateral Renovascular or Renal Disease

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ABSTRACT A study of 44 hypertensive patients with unilateral renovascular or renal parenchymal disease is presented All patients underwent corrective surgery Out of the 44 operated patients, five did not participate in the follow up examination The remaining 39 patients constitute the study population The effects of surgery on the hypertensive state could be evaluated in 35 patients, whereas four died less than two months after the operation Follow up studies were carried out at 8–60 months after the operation The average period of observation was 32 months, 24 patients were observed for more than two years As a group, the patients had severe hypertension with extensive target organ damage and widespread atherosclerosis A fairly rigorous selection process was applied and an unsatisfactory response to medical management was considered a point of major importance In the majority of cases renovascular lesions were atherosclerotic with only two cases of fibromuscular dysplasia Unilateral nephrectomy was performed in 32 patients whereas seven underwent reconstructive vascular surgery Out of 35 patients 22 (63%) were cured, 8 (23%) improved and 5 (14%) unaltered A gratifying regression of hypertensive lesions in target organs was observed in patients who were cured or improved by surgery The frequency and severity of postoperative complications were related to the presence of extrarenal vascular disease

Key words renal hypertension renovascular hypertension

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Unilateral renovascular or renal parenchymal diseases are common causes of secondary hypertension The operative results have been reported in many studies (4 6 7 12 22) The frequency of cure improvement or failure varies considerably with the composition of the patient populations and with the selection criteria

We report our experience in surgical treatment of renal hypertension Important aspects are the effects on BP the regression of hypertensive cardiovascular lesions and the principles underlying patient selection for operation

PATIENTS AND METHODS

During 1970–75 44 hypertensive patients underwent surgery for renal or renovascular disease In 5 patients the postoperative course could not be assessed 2 left the country and 3 declined the offer The remaining patient population comprised 14 females and 25 males The mean age for the women was 46 years and for the men 50 years The majority of the male patients were in the age group 41–60 years with a more even age distribution of the female patients

Duration of disease Ten patients had been hypertensive for more than two years In 21 patients hypertension had been present for ½–2 years and 8 patients had had a known hypertensive disease for less than 6 months

Measurement of blood pressure Repeated measurements were obtained during admission to the hospital Values are given as the average of no less than three separate readings obtained in the morning with the patients supine and at rest Diastolic pressures were read at the point at which the Korotkoff sounds disappear (phase V)

The preoperative group average before drug treatment was 225 mmHg (S D ±28) systolic and 137 mmHg (S D ±19) diastolic

Hypertensive lesions in target organs Cardiac enlargement or left ventricular hypertrophy as detected by

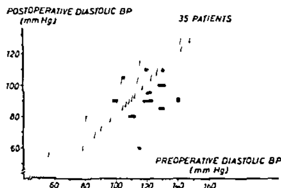


Fig 1 Diastolic BP before and after corrective surgery in 35 hypertensive patients The hatched area illustrates a decrease of 20 mmHg

chest X ray and/or ECG were present in 31 out of 39 patients Retinal changes were classifed by an ophthalmologist Hypertensive retinal changes grades III or IV (Keith Wagener and Barker scale) were present in 21 patients (54%) As a reflection of a policy of selection only 2 patients had a glomerular filtration rate (GFR) lower than 40 ml/min×1 73 m² Prior to operation 16 patients (41%) had shown manifestations of cerebrovascular disease Further details are given in Table II Nine patients (23%) had signs of arterial insufficiency of the lower extremities Two male patients had clear-cut evidence of coronary artery disease (myocardial infarction and angina pectoris respectively)

Response to treatment with antihypertensive drugs Prior to surgical intervention 36 out of 39 patients were treated with hypotensive drugs In 35 cases combined ι with 2–4 drugs was used A diastolic BP lower ι 100 mmHg was achieved in only 2 patients

sessment of renal function before and after operation GFR was assessed by measurement of ^{51}Cr EDTA clearance (3) The estimated residual postoperative GFR in patients scheduled for unilateral nephrectomy was calculated from preoperative measurements of ^{51}Cr EDTA clearance (total renal function) and radioisotope renography (fractional share of total renal function for each of the two kidneys) Details are given in an earlier publication (19)

In 28 patients undergoing unilateral nephrectomy postoperative measurements of ^{51}Cr EDTA clearance were performed and the measured values were compared to the preoperatively estimated residual GFR

Renal radiology and radioisotope renography The diagnosis of unilateral renal or renovascular disease was based upon radioisotope renography ι v pyelography and renal angiography Only 4 patients had unilateral renal parenchymal disease Two patients had unilateral chronic pyelonephritis one had a renal infarct and one patient had sequelae of unilateral kidney trauma Out of 35 patients with renovascular disease 33 had atherosclerotic lesions and 2 fibromuscular dysplasia

The criteria for evaluation of radioisotope renograms in terms of lateralization have been published elsewhere (10) As far as ι v pyelography is concerned the term lateralization is used to indicate the presence of unilateral abnormalities as recorded in the radiologist s report

Catheterization of the renal veins Renal vein catheterization was carried out in all patients Correct placing of the catheters was ensured by means of fluoroscopy and measurements of oxygen saturation in blood samples Samples were drawn simultaneously from both renal veins and from a systemic artery alternatively samples from the distal part of the inferior vena cava were used for reference Determinations of plasma renin concentration in blood samples were performed as described by Giese et al (9)

The following criteria were laid down for lateralization of renin secretion 1) The plasma renin concentration in venous blood from the affected kidney should be at least 1 5 times higher than from the contralateral kidney 2) There should be a statistically significant renin gradient across the affected kidney but not across the contralateral

General schedule for diagnostic studies We have used the following sequence of diagnostic studies in hypertensive patients A meticulous clinical examination aided by specific studies of target organs should provide fundamental data for evaluating the need for etiologic studies Radioisotope renography was performed as a non invasive initial renal study With a lateralizing renogram at hand the next step might be either ι v pyelography or renal angiography for detection or exclusion of renal or renovascular lesions Measurements of GFR were performed as a routine Renal vein catheterization was carried out in patients with radiological evidence of renal or renovascular disease accessible to surgical treatment

Indications for surgery The decision to operate upon a given patient was made by a panel of physicians and surgeons after presentation of all clinical data and diagnostic studies Particular attention was paid to the individual response to treatment with antihypertensive drugs

Surgical procedures Thirty two patients underwent unilateral nephrectomy 13 of these had unilateral renal artery occlusion and 4 unilateral renal parenchymal disease In 3 patients reconstructive vascular surgery proved technically impracticable One patient underwent secondary nephrectomy after primary autotransplantation with subsequent thrombosis of the renal artery

The reasons for the choice of unilateral nephrectomy rather than reconstructive vascular surgery in the remaining 11 patients were the presence of extensive atherosclerosis and/or the integrated clinical evaluation of the patient as a high risk case

Seven patients had reconstructive vascular surgery with pre and postoperative anticoagulation treatment In two patients autotransplantation was carried out and one had a splenorenal anastomosis

Reconstructive vascular surgery was mainly applied in patients with less extensive stigmata and of course technically accessible renovascular lesions

Plan for follow-up study The patients were studied during a 36-hour admission to the Department of Internal Medicine A general clinical examination was carried out BP was measured and specific studies to determine the extent of hypertensive cardiovascular lesions were performed The average interval from surgery to follow up

Table I *Retinal lesions in 34 hypertensive patients before and after corrective surgery*

	Fundi grade (Keith Wagener & Barker scale)				
	0	I	II	III	IV
Before operation	0	2	14	17	1
After operation	7	14	13	0	0

was 32 months (range 8–60) 24 out of 35 patients were examined after an interval of 2 years or more

Criteria for assessment of the effects of surgical treatment The following criteria were accepted

Cured Normal BP according to Master s criteria (14) without antihypertensive medication or with low dosage diuretic therapy

Improved Decrease of diastolic BP by 20 mmHg or more in patients on antihypertensive medication in a lower dosage than required preoperatively together with regression of hypertensive stigmata

Not significantly improved Criteria for cure or improvement not fulfilled

RESULTS

Peroperative and early postoperative mortality

One patient died of irreversible ventricular fibrilla tion during operation for stenosis of the abdominal aorta and unilateral renovascular disease Two high risk patients died less than 2 months after surgery one of mesenteric arterial occlusion the other of cardiac decompensation One fatal cerebrovascular attack occurred in the early postoperative phase

Blood pressure

A comparison of pre and postoperative diastolic blood pressures is given in Fig 1 Great care has been taken to permit meaningful comparisons between the BP readings obtained prior to operation and at the time of the follow up study respectively in particular with respect to medication

Thirty out of 35 patients (86%) achieved a definite benefit 22 (63%) became normotensive and 8 (23%) were improved Five patients (14%) were not significantly improved after the operation

Regression of hypertensive cardiovascular lesions after surgical intervention

Retina At the time of follow up not a single patient presented severe retinal lesions (grades III and IV) Details are given in Table I

Heart Cardiac enlargement or left ventricular

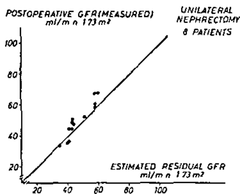


Fig 2 Relation between estimated residual GFR and *measured GFR after unilateral nephrectomy in 28 hypertensive* patients with unilateral renovascular or renal parenchymal disease

hypertrophy was found in only 2 patients at follow up Both of these had an unsatisfactory BP response

Brain 7 patients had cerebrovascular attacks during the interval between operation and follow up All except one had been similarly affected prior to the operation A comparison of the recorded manifestations of cerebrovascular disease before and after operation is given in Table II

Postoperative renal function

No case of postoperative renal insufficiency occurred A comparison between the preoperatively estimated residual GFR and the measured residual GFR at follow up in 28 unilaterally nephrectomized patients is shown in Fig 2

Late deaths

Three late deaths were recorded at 14 30 and 42 months respectively Two patients had acute

Table II *Cerebrovascular disease in 39 hypertensive patients before and after corrective surgery*

	Prior to operation (1–72 mo)	After operation (0–60 mo)
Cerebrovascular accidents	10	5
Transitory ischemic attacks	3	2
Hypertensive encephalopathy	3	0
Total	16	7

Table III *Operative results in relation to renal/renovascular pathology in 39 hypertensive patients*

	Unilateral renal artery stenosis	Unilateral renal artery occlusion	Unilateral parenchymal disease	Total
Cured	13	7	2	22
Improved	5	2	1	8
Not significantly improved	2	2	1	5
Deaths	2	2	0	4
Total	22	13	4	39

myocardial infarction both were classified as improved One patient classified as not significantly improved had a fatal stroke

Results related to anatomical diagnosis surgical procedure and age

The relation between the anatomical diagnosis and the outcome of operation is shown in Table III There is no impressive difference between the results obtained in the three categories listed

The results obtained with reconstructive vascular surgery and unilateral nephrectomy respectively are given in Table IV No failures were recorded in the highly selected group of patients undergoing vascular reconstruction At the time of the operation 18 patients were above 50 years of age The results obtained in this particular subgroup are detailed in Table V for different age groups Eight out ten patients above 60 years of age were cured or roved

Pyelography renography and renal venous renin studies versus outcome

The results of i v pyelography radioisotope renography and renal venous catheterization were available for comparison with the follow up assessment in 31 patients as shown in Fig 3

The preoperative studies showed overall lateralization in 19 patients, 3 of these had no significant improvement Six patients had unilateral abnormalities at i v pyelography and radioisotope renography but no lateralization of renin secretion Four of these were cured or improved Six patients with no recorded unilateral abnormality at intravenous pyelography but lateralization of renin secretion and abnormal renography, became normotensive

DISCUSSION

Every unit devoting part of its resources to the diagnosis and treatment of renal and renovascular hypertension is continually faced with the question of the value of case finding in hypertensive renovascular disease and the choice between medical management and surgical intervention

To our knowledge the ideal study comparing the outcome of drug treatment and corrective surgery respectively in strictly comparable groups of hypertensive patients with renovascular disease has not been carried out so far However the prospective studies of Hunt et al (11) in a large series of patients have provided important information Their basic policy was to prefer medical manage

Table IV *Operative results in 39 hypertensive patients listed according to surgical procedure*

	Reconstruction	Unilateral nephrectomy	Total
Cured	5	17	22
Improved	1	7	8
Not significantly improved	0	5	5
Deaths	1	3	4
Total	7	32	39

Table V *Results of corrective surgery in 18 hypertensive patients above 50 years of age*

Age (y)	50–54	55–59	60–65	>65	Total n	Total %
Normotension	5	1	5	1	12	66
Improved	1	0	2	0	3	17
Failures	0	1	2	0	3	17
Total	6	2	9	1	18	100

INTRAVENOUS PYELOGRAPHY	☐	☐	☐
RADIOISOTOPE RENOGRAPHY	☐	☐	☐
RENAL VENOUS RENIN	☐	☐	☐
NORMOTENSIVE OR IMPROVED	16	4	6
NOT SIGNIFICANTLY IMPROVED	3	2	0

☐ LATERALIZATION

Fig 3 Results of pyelography renography and renal venous renin studies versus outcome

ment in the first instance Most patients were submitted to surgical treatment only after an unsatisfactory trial of antihypertensive drug therapy The data provided strong evidence in favour of surgical intervention

Our experience was similarly obtained in a population of patients most of whom had shown an unsatisfactory response to medical management or outright refractoriness Furthermore there was a high prevalence of extensive atherosclerosis and hypertensive vascular lesions About one half of the patients were above 50 years of age Against this background the results are encouraging they show a gratifying rate of successful interventions with 86% of the patients achieving normotension or a significant decrease of BP together with an impressive regression of retinal and cardiovascular lesions Our results in older patients support the attitude of Dean and Foster (5) there is no background for rigid application of age limits in the selection process

Based upon previous experience (20) our criteria for evaluation of the outcome have been rather strict While this approach has obvious advantages it may tend to obscure the fact that a number of patients classified as not significantly improved still had the considerable benefit of being easier to manage by means of drugs than before the operation

Renal atherosclerotic lesions constitute only one of many manifestations of a more or less generalized vascular affection Renal vascular reconstruction or unilateral nephrectomy can of course only decelerate the progress of extrarenal atherosclerosis by way of cure or improvement of hypertension Hopefully the intervention in this way may prevent vascular catastrophes in vital areas such as the brain Our results do lend support to this concept

The prognostic importance of extrarenal atherosclerotic lesions has been pointed out by several authors (7 8 21) An overview of the causes of early and late postoperative deaths in our group of patients gives great support to this line of thought as does the information on the pre and postoperative occurrence of cerebrovascular attacks

The surgical procedure was unilateral nephrectomy in the majority of our cases Whereas this approach is at variance with the time honoured concept emphasizing the preservation of nephrons it has definite advantages in patients with severe hypertension extensive atherosclerotic lesions in the renal artery aorta and elsewhere and—not infrequently—a rather decrepit general state of health It should be pointed out that about half of the nephrectomies were performed in cases of unilateral renal parenchymal disease or renal artery occlusion

This approach requires however very careful attention to the preoperative estimation of expected postoperative residual renal function With the techniques employed here we had no unpleasant experience in that respect As expected (18) postoperative hypertrophy of the remaining kidney occurred in many cases leading to a higher postoperative GFR than was estimated from the preoperative studies

The prognostic value of renal venous renin studies in terms of prediction of operative results has been a subject of interest to many investigators (11 12 13 15) In our decision making the presence of lateralization of renin secretion has carried a distinct weight in favour of surgery whereas preference has been given to medical management in several patients presenting non lateralizing renal venous renin studies The only incontrovertible criterion for the diagnosis renal or renovascular hypertension is the documented abolition or definite improvement of the hypertensive state following technically successful surgical intervention Thus the diagnosis remains unsettled in the non operated patients

In view of these circumstances the data presented in Fig 3 are not suitable for the derivation of predictive values It is however of note that renal venous renin studies can provide false negative results i e no clear cut lateralization of renin secretion in patients subsequently cured after intervention on the affected kidney as reported by other investigators (12 13 15) In our series of operated patients 22 out of 25 patients with lateralization of

renin secretion were cured or improved after surgery

The attitude adopted towards the strategy of diagnostic work up in hypertensive patients varies rather widely Recent reports (1 16 17) have presented data throwing doubt on the value of identification of renovascular disease in hypertensive populations and some authors (2 23) maintain that there is no need to identify the occasional case of renal/renovascular hypertension in the context of population screening Others would deem it wise to apply simple screening methods at least in hospitalized hypertensive patients We would like to emphasize that there is a world of difference between the simple expedient of performing a radioisotope renography and the wholesale application of a battery of invasive and costly investigations In our view the simple renographic approach is a reasoned one (10)

CONCLUSION

The results obtained in the present follow up study would support the general concept that it is certainly worthwhile to identify the presence of unilateral renovascular disease or unilateral contracted kidney in a hospital population of hypertensive patients

REFERENCES

1 Berglund G A homogenously investigated highly selected hypertensive population Acta Med Scand 194 87 1973
2 Berglund G Andersson O & Wilhelmson L Prevalence of primary and secondary hypertension studies in a random population sample Br Med J 2 554 1976
3 Brøchner Mortensen J A simple method for the determination of glomerular filtration rate Scand J Clin Lab Invest 30 271 1972
4 Buchardt Hansen H J & Nerstrøm B Kirurgisk behandling af renovaskulær hypertension Ugeskr Læger 136 413 1974
5 Dean R H & Foste J H Surgical management of renovascular hypertension in older patients Med Clin North Am 61 643 1977
6 Ernst C B Stanley J C Marshall F F & Fry W J Autogenous saphenous vein aortorenal grafts Arch Surg 105 855 1972
7 Foster J H Marxwell M H Franklin S S Bleifer K H Trippel O H Julian O C DeCamp P T & Varady P T Renovascular occlusive disease JAMA 231 1043 1975
8 Franklin S S Young J D Maxwell M H Foster J H Palmer J M Cerny J & Varady P D Operative morbidity and mortality in renovascular disease JAMA 231 1148 1975
9 Giese J Jorgensen M Nielsen M D Lund J O & Munck O Plasma renin concentration measured by use of radioimmunoassay for angiotensin I Scand J Clin Lab Invest 26 355 1970
10 Giese J Mogensen P & Munck O Diagnostic value of renography for detection of unilateral renal or renovascular disease in hypertensive patients Scand J Clin Lab Invest 35 307 1975
11 Hunt J C Sheps S G Harrison E G Strong C G & Bernatz P E Renal and renovascular hypertension Arch Int Med 133 988 1974
12 Juncos L I Strong C G & Hunt J C Prediction of results of surgery for renal and renovascular hypertension Arch Intern Med 134 655 1974
13 Marks L S Maxwell M H Varady P D Lupu A N & Kaufman J J Renovascular hypertension does the renal vein renin ratio predict operative results? J Urol 115 365 1976
14 Master A M Garfield C I & Walters M B Normal blood pressure and hypertension Lea & Febiger Philadelphia 1952
15 Maxwell M H Marks L S Lupu A N Canill P J Franklin S S & Kaufman J J Predictive value of renin determinations in renal artery stenosis JAMA 238 2617 1977
16 McNeil B J & Adelstein S J The value of case finding in hypertensive renovascular disease N Engl J Med 293 221 1975
17 McNeil B J Varady P D Burrows B A & Adelstein S J Cost effectiveness calculations in the diagnosis and treatment of hypertensive renovascular disease N Engl J Med 293 216 1975
18 Mogensen P Munck O Tønnesen K H & Wolf H Compensatory renal hypertrophia in patients undergoing unilateral nephrectomy Scand J Urol Nephrol 11 155 1977
19 Mogensen P Rossing N & Giese J Glomerular filtration rate measurement and ^{131}I hippuran renography before unilateral nephrectomy Scand J Urol Nephrol 6 228 1972
20 Munck O Faarup P Gammelgaard P A Ladefoged J Mathiesen F R & Pedersen F Characteristics of renovascular hypertension Scand J Clin Lab Invest 22 288 1968
21 Simon N Franklin S S Bleifer K H & Maxwell M H Clinical characteristics of renovascular hypertension JAMA 220 1209 1972
22 Stanley J C & Fry W J Renovascular hypertension secondary to arterial fibrodysplasia in adults Arch Surg 110 922 1975
23 Werko L Underlag för medicinska beslut Lakartidningen 72 4487 1975

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Glomerulonephritis and Exposure to Organic Solvents

A Case Control Study

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ABSTRACT Fifty patients with biopsy proven glomerulonephritis and 100 sex and age-matched controls (50 patients each with non glomerular renal disease or acute appendicitis) were asked by questionnaire and a telephone interview whether they had been exposed to organic solvents The questioning and the evaluation of the exposure, if any, was made without knowing the diagnosis of the interviewee Fifty per cent of the patients with glomerulonephritis reported more than slight exposure, but only 20% of the controls Exposure to organic solvents may often play a role in the causation of glomerulonephritis

Key words glomerulonephritis organic solvents hydrocarbons occupational disease

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Based on observations in a few patients it has been suggested that exposure to vapours of organic solvents and fuels may cause glomerulonephritis both idiopathic (1 8 9 24 25 26) and as part of Goodpasture s syndrome (2 7 14 18 28 29) But in view of the rarity of such cases it might be argued that such an association may be simply coincidental On the other hand the finding by Zimmerman et al (33) that the vast majority of their patients with terminal renal failure caused by glomerulonephritis had been exposed to vapours of various organic solvents lends support to the connection being causal rather than casual The study by Lagrue et al (19) pointed in the same direction

If true such a causal relationship would have important implications in the management of glomerulonephritis the dominating cause of chronic renal failure to-day We therefore thought it urgent to find out whether we could reproduce the findings by the above mentioned authors

PATIENTS AND METHODS

Since Nov 1973 adults admitted to the Department of Nephrology in Lund Sweden with clinical signs of glomerulonephritis have entered a research programme including needle biopsy of the kidney Patients with contracted kidneys in association with a glomerular filtration rate below 30 ml/min were excluded The first 56 consecutive patients in whom the diagnosis was verified by renal biopsy were regarded as acceptable for the study but six of them died before we attempted to contact them This left 50 patients for the present study In two patients the glomerulonephritis was secondary to a systemic disease in the others it was primary The patient series included all of the most common histologic types of the disease (Table I)

For each patient with glomerulonephritis there were two sex and age matched (±5 years) controls One of each control pair was a patient with a non glomerular renal disease who was admitted within the same period and selected from the files of the Department of Nephrology The diagnoses of the patients in this control group are given in Table II Of the 56 patients selected originally six died before any attempt was made to contact them The other control was a patient who had been operated upon at the Department of Surgery Lund within the same period because of acute appendicitis The diagnosis was

Table I Histologic classification in the glomerulonephritis group

All biopsy specimens were studied by light 46 by immunofluorescence and 30 by electron microscopy

	N
Proliferative	11
Exudative	1
Focal proliferative	19
Extracapillary	2
Membranoproliferative	4
Epimembranous	1
Minimal changes	6
Focal sclerosis	2
Not classifiable	4
Total	50

Table II *Diagnoses of control patients with non glomerular renal disease*

	N
Calculi of the urinary tract	13
Pyelonephritis acute and/or chronic	11
Polycystic kidney disease	12
Congenital hypoplasia of the kidney(s)	4
Congenital hydronephrosis	3
Renal tubular acidosis	2
Medullary sponge kidney	2
Other congenital defects	3
Total	50

verified by microscopic examination of the appendix Patients with raised serum creatinine proteinuria or hypertension were excluded Of the 52 patients selected originally two had died

Apart from the deaths prior to the investigation there were no drop-outs The ages of the glomerulonephritis patients ranged from 17 to 65 years (median 32) In each control group the corresponding figures were 16–66 and 31 respectively Each group consisted of 34 men and 16 women

The serum creatinine concentrations at the time of renal biopsy in the glomerulonephritis group ranged from 60 to 1300 μmol/l (median 100) and in the non glomerular disease group from 70 to 450 μmol/l (median 90) The upper limit of the normal range for men at our laboratory is 115 μmol/l

All participants received a questionnaire with questions about occupational and spare time activities possibly involving exposure to organic solvents or fuels They were also interviewed by telephone by an occupational chemist who had access to the patient s answers in the questionnaire but was unaware whether the interviewee was patient or control

On the basis of the information obtained in this way the exposure up to the time of the renal biopsy of the patient and his/her controls was expressed as a work level × time score For each activity attended by exposure to solvents the weekly number of hours of exposure was multiplied by the number of years the activity had been practised The duration of the exposure was multiplied by an exposure intensity factor of 0 5 1 or 2 Only estimated time in actual contact with organic solvents was used when the score was calculated The actual working conditions were taken into account when choosing the factor in an individual person and activity

Heavy exposure (intensity factor 2)

Occupational house painting indoor (5 13) Industrial spray painting without protection devices (15) Carpet and floor laying (17 27) Production of paint and glue (32) Polyester plastic application (3 10)

Moderate exposure (intensity factor 1)

Non-occupational house painting indoor Spray painting with protection devices (4 30) Industrial degreasing of metal Printing work (22 31) Occupational gluing (16) Anaesthesiologic work (11 12) Dry cleaning

Slight exposure (intensity factor 0.5)

Outdoor painting Motor repairing handling of petrol Hobby gluing drawing with filter tipped pens

Example A patient who had for 4 years worked half time doing indoor house painting was allotted the following score 20 (hours per week) ×4 (years) ×2 (exposure level)=160

The score was decided jointly by the interviewer (A Wilstermann) and one of us (S S) neither of whom was aware of the group to which the individuals belonged

Drs C Brun F Jorgensen and S Larsen examined the renal biopsy specimens by light electron and immunofluorescence microscopy respectively

RESULTS

The scores ranged from 0 to 1440 (median 14) in the glomerulonephritis group from 0 to 826 in the non glomerular disease group and from 0 to 560 in the appendicitis group In each control group the median was one Fifty per cent of the patients with glomerulonephritis and 20% of the controls had a score above 10 (Fig 1)

The patient material was analysed as matched triplets according to Miettinen (20 21) When individuals with a score above 10 were considered as exposed and individuals with a score below 10 as non exposed the rate ratio (relative risk) was 3 9 The difference between the groups was statistically significant (Table III) A high relative risk was present both in women (rate ratio 5 3) and men (rate ratio 3 8) The relative risk was the same whether it

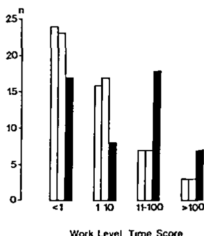


Fig 1 Exposure to organic solvents ■=Patients with glomerulonphritis □=controls (left non glomerular renal diseases right acute appendicitis)

Table III *Distribution of solvent exposed (exposure score above 10) and non exposed (exposure score below 10) individuals in 50 triplets*

Each triplet consists of one patient with glomerulonephritis and two sex and age matched controls (one with non glomerular renal disease one with acute appendicitis) Point estimate of rate ratio=3 9 95% confidence interval=1 9–8 1 χ^2 -13 2 p=0 0003 (two-tailed)

	No of triplets in which the controls comprised		
	2 exposed	1 exposed 1 non exposed	2 non-exposed
No of triplets in which the glomerulonephritis patient was			
Exposed	0	8	17
Non-exposed	3	6	16

was based on the score for the last five years only or for the total duration of exposure

No difference was found between the degree of exposure in the two control groups An analysis of the matched control pairs (non glomerular renal disease versus appendicitis) gave a rate ratio of 1 0 The relative risk in different exposure score ranges showed a tendency towards a dose–respon e relationship (Table IV)

The patients with glomerulonephritis and the controls with non glomerular renal disease had been admitted from a larger area than the controls with acute appendicitis who were residents of the town of Lund or one of its nearby communities As Lund is a university town individuals with low exposure might have been in excess in the appendicitis group But in the glomerulonephritis group exposure to solvents was reported slightly more often by patients living in Lund or its vicinity than by those living outside this area

Table IV *Rate ratio between patients with glomerulonephritis and controls at different degrees of exposure to organic solvents*

As the number of individuals with a score above 100 was too small for a sound statistical calculation we chose a classification slightly different from that used in the figure

Exposure score	Patients	Controls	Rate ratio
<1	17	46	1 0
1–10	8	34	0 6
11–50	14	13	2 9
>50	11	7	4 2
Total	50	100	

DISCUSSION

Judging from the findings in our series a relatively larger number of patients with glomerulonephritis than with various non glomerular renal diseases or acute appendicitis had been exposed to organic solvents Patients with glomerulonephritis may have searched their memories more thoroughly than the controls But the purpose of the study was not obvious to the participants identical questionnaires were used and what is possibly most important the interviewer whose thorough questioning provided most of the information obtained did not know whether the interviewee was patient or control These precautions may have been insufficient to exclude any memory bias completely But in the individuals with a score above 10 more than 90% of the exposure was occupational and in view of the thoroughness of the interview such exposure could hardly have escaped detection to any greater degree

The study was retrospective and the patients with glomerulonephritis had been selected before any association between exposure to solvents and glomerulonephritis had ever been suspected For the group with acute appendicitis a selection bias can also be excluded as only their names and dates of birth were indicated in the files from which the subjects of the group were chosen

In the selection of the patients with non glomerular renal disease their records had to be examined thoroughly to exclude those with findings suggesting glomerulonephritis Any possible exposure to solvents was not mentioned in their records at that time but awareness of their occupations might unintentionally have favoured a rejection from the study We feel however that to avoid this bias we were more likely to accept controls with an occupation known to be associated with exposure to organic solvents than to exclude them because of discrete or equivocal signs of glomerular disease The similar frequency of exposure to solvents in the appendicitis group as in the non glomerular renal disease group indicated that any selection bias had been only negligible

Efforts were made to persuade patients and controls to participate in the study and to track indi

viduals who had moved out of our area Thus the only cause of non participation was death which could hardly have affected the results

The controls might have been awarded lower scores if they had had sick leaves for longer periods than the patients with glomerulonephritis But this was not so the sick leaves were few and short in all three groups no patient had retired and none were being treated with hemodialysis at the time of renal biopsy

A hypothetical but most improbable explanation of our findings is that hydrocarbons offer protection against acute appendicitis and non glomerular renal diseases As most solvents are nephrotoxic to experimental animals (6) one should rather suspect that exposure to solvents could worsen any renal disease We therefore feel that if the controls in the non glomerular renal disease group differed in degree of exposure from the average population they should rather be more exposed than less

An estimate of the degree of exposure from information obtained by a mailed questionnaire and a telephone interview may be uncertain in several aspects Published data on levels of organic solvents in the environmental atmosphere in association with different occupations and hobbies are scanty and it may be incorrect to use such data because of the wide variations of such factors as ventilation and physical work load However these ts could only obstruct an attempt to show a erence between patients and controls but not induce a systematic error

Although every bias may not have been avoided completely we think that if any it cannot explain the great difference between exposure to *solvents in patients and controls* Thus exposure to organic solvents in some way seems to be associated with glomerulonephritis Hydrocarbons could either be a direct cause of glomerulonephritis or they could have a harmful influence on its *course*

Exposure to organic solvents is common in Sweden as well as in other industrial countries The general exposure level is suggested by the finding that more than 20% of our controls reported an exposure equivalent to more than ten 40-hour working weeks of continuous exposure to vapours of hydrocarbons (>10 points) As glomerulonephritis is uncommon there must obviously be also other factors of importance A concurrence between a streptococcal infection and hydrocarbon toxicity has been suggested in some patients (23) However most patients with glomerulonephritis have no evidence of streptococcal infection in their history or clinical data

Glomerulonephritis the major cause of terminal renal failure to day is a catastrophe for the patient and an economic burden for society Recognition of an association between exposure to hydrocarbons and glomerulonephritis thus calls for practical measures Patients with glomerulonephritis should as far as possible avoid exposure to organic solvents Observations at our department indicate that elimination of exposure may have a favourable effect on the disease Glomerulonephritis should be recognized legally as an occupational disease and our findings indicate the need for general measures to decrease occupational exposure

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REFERENCES

1 Anderson K Acute nephritis due to turpentine absorbed by the skin Br Med J 3 881 1912
2 Beirne G J & Brennan J T Glomerulonephritis associated with hydrocarbon solvents mediated by antiglomerular basement membrane antibody Arch Environ Health 25 365 1972
3 Bergman K Styrenexposition i plastbåtsindustri Arbete och Halsa 3 1 1977 (ISBN9 7464-002 X ISSN 0346-7821)
4 Berseus B Sprutmålares exposition för losnings medelsångor och pigment In Hogstedt C Losningsmedel—tekniska och medicinska aspekter p 33 Arbetarskyddsstyrelsen Stockholm 1977
5 Bobjer O Byggnadsmålares arbetsmiljo—pilot studie Arbetarskyddsstyrelsen Stockholm 1978
6 Browning E Toxicity and metabolism of industrial solvents Elsevier Amsterdam 1965
7 D Apice A J F Kincaid Smith P Becker G J Loughhead M G Freeman J W & Sands J M Goodpasture s syndrome in identical twins Ann Intern Med 88 61 1978
8 Ehrenreich T Yunis S L & Churg J Membranous nephropathy following exposure to volatile hydrocarbons Environ Res 14 35 1977
9 Glaeser J A *Kleine Mitteilungen* Zschr Klin Med 21 377 1892
10 Gotell P Axelson O & Lindelof O Field studies on human styrene exposure Work Environ Health 9 76 1972
11 Gotell P & Ståhl R Halotan-exposition hos narkosskoterskor Lakartidningen 69 6179 1972

12 Gothe C J Örum P & Hallen P Exposure to anesthetic gases and ethanol during work in operating rooms Scand J Work Environ Health 2 96 1976
13 Hallin N Arbetshygieniska problem vid målen arbete—losningsmedelsångor från fargprodukter Byggförlaget Stockholm 1975
14 Heale W F Matthiesson A M & Niall J F Lung haemorrhage and nephritis (Goodpasture s syndrome) Med J Aust 3 355 1969
15 Ivegärd T Arbetsmiljoproblem vid ytbehandling inom traindustrin Ergolab Stockholm 1975
16 Johansson B & Larsson O Arbetshygieniska risker vid limning av PVC och ABS-ror Bygghalsan Stockholm 1975
17 Jonsson K & Kirudd H Akrylplast—en arbetsmiljostudie vid laggning av fogfria golv på metylmetakrylatbas Arbetarskyddsstyrelsen Stockholm 1977
18 Klavis G & Drommer W Goodpasture Syndrom und Benzineinwirkung Arch Toxicol 26 40 1970
19 Lagrue G Kamalodine T Guerrero J Hirbec G Zhepova F & Bernaudin J F Nephropathies glomerulaires primitives et inhalation de substances toxiques J Urol Nephrol 83 323 1977
20 Miettinen O S Individual matching with multiple controls in the case of all-or none respon es Biometrics 25 339 1969
21 — Matching and design efficiency in retrospective studies Biometrics 26 75 1970
22 Övrum P Hultengren M & Lindquist T Exposition för Toluen och upptag i kroppen vid arbete i djuptrycken Arbete och Halsa 4 1 1977 (ISBN 91 7464-003 8 ISSN 0346-7821)
23 Ravnskov U Exposure to organic solvents—a missing link in poststreptococcal glomerulonephritis? Acta Med Scand 203 351 1978
24 Reinhard Ein Fall von Terpentinintoxication in Folge Einathmens von Terpentinol Dtsch Med Wochenschr 13 256 1887
25 Ridder Terpentinolvergiftung mit Nierenschadigung durch ausserliche Anwendung des Ols Dtsch Med Wochenschr 2 1369 1923
26 von Scheele C Althoff P Kempi V & Schelin U Nephrotic syndrome due to subacute glomerulonephritis—association with hydrocarbon exposure? Acta Med Scand 200 427 1976
27 Schutz A Losningsmedelsförgiftningsriskerna i golvlaggningsyrket Yrkesmedicinska kliniken Lund 1968
28 Seeliger K & Huland H Kasuistischer Beitrag zur Atiologie des Goodpasture Syndroms Med Klin 68 437 1973
29 Sprecace G A Idiopathic pulmonary hemosiderosis Am Rev Resp Dis 88 330 1963
30 Steby M Expositionsförhållanden vid bil- och industrilackering In Högstedt C Losningsmedel—tekniska och medicinska aspekter Arbetarskyddsstyrelsen Stockholm 1977
31 Stokholm J & Cohr K H Toluen litteratur oversigt forekomst på danske arbejdspladser og forslag til videre undersøgelser Arbejdstilsynet København 1975
32 Ulfvarsson U A field investigation of chemical hazards in the paint industry IVth National Symposium on the control of air pollution in the working environment Stockholm 1977
33 Zimmerman S W Groehler K & Beirne G J Hydrocarbon exposure and chronic glomerulonephritis Lancet 2 199 1975

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Acute Glomerulonephritis and Exposure to Organic Solvents in Father and Daughter

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ABSTRACT A man and his daughter had acute glomerulonephritis the daughter eight years after the father Both were exposed to vapours of organic solvents shortly before the onset of nephritis

Key words glomerulonephritis organic solvents hydrocarbons

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Exposure to organic solvents or fuels has been suspected of being a causal factor in some patients with Goodpasture s syndrome (1 2 4 5 9 10) and in a great number of patients with chronic non systemic glomerulonephritis (6 9 11) and acute poststreptococcal glomerulonephritis (7) The use of organic solvents is widespread infectious diseases are ubiquitous but the above diseases are uncommon or rare There is obviously a reason to search for other contributing factors

THE FATHER

A 30-year-old man had a nephrotic syndrome in 1967 During the two preceding months he had been exposed indoors to vapours of "thinners and petrol for eight hours a day Ten days before admission his legs suddenly swelled His urine contained numerous erythrocytes and 14 [illegible] protein Serum creatinine and urea were normal An i v pyelography and renal phlebography showed nothing unusual except for a moderate enlargement of the kidneys The ASO titer was negative on admission and three weeks later as was a test for antinuclear antibodies A light microscopic examination of a kidney biopsy specimen showed membranous glomerulonephritis The patient recovered after a few months treatment with azathioprine and prednisolone Exposure to solvents and fuels was discontinued after the acute onset of the disease At follow up seven years later there was no clinical or laboratory evidence of renal disease

THE DAUGHTER

Eight years later a 10-year-old daughter of the above mentioned patient had an acute glomerulonephritis 17 days after throat infection with β hemolytic streptococci The serum creatinine was 170 μmol/l ASO titer 720 C3 24% of the normal mean and C4 within normal limits During the interval between the streptococcal infection and the acute onset of the nephritis she was exposed to vapours of thinners Her history and data were recently published together with those of nine other patients with poststreptococcal glomerulonephritis who had been exposed to volatile hydrocarbons prior to the onset of the nephritis (7)

DISCUSSION

The first observation pointing to a genetic factor behind solvent associated glomerulonephritis was probably made by Glaeser in 1892 (3) Two sisters who had for five weeks lived in an atmosphere polluted with turpentine vapours were admitted one two weeks after the other with an acute nephrotic syndrome One of the sisters had had an infection of a tooth a week earlier Recently D Apice et al (2) reported Goodpasture s syndrome in identical twins exposed to volatile hydrocarbons The present cases lend further support to the possibility that renal susceptibility to the nephritogenic properties of solvents may be inherited

That the inherited component is an immunoresponse gene is possible In a study of 127 patients with glomerulonephritis we found however no significant differences in the mean white blood cell differential count or in the mean plasma or serum concentration of IgG IgA IgM C3 C4 Clq C3 proactivator in properdin or in Clq reacting substances between exposed and unexposed patients (8)

An interesting observation was that the daughter had clear cut acute poststreptococcal glomerulonephritis whereas the father had an acute nephrotic syndrome without evidence of streptococcal etiology Obviously individuals with a similar

hereditary background may acquire different types of glomerulonephritis depending on exogenous factors In other words the pathogenesis of glomerulonephritis may include microbiologic environmental and hereditary components Variations in these factors may determine the clinical and histologic features of the disease

ACKNOWLEDGEMENT

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REFERENCES

1 Beirne G J & Brennan J T Glomerulonephritis associated with hydrocarbon solvents mediated by antiglomerular basement membrane antibody Arch Environ Health 25 365 1972
2 D Apice A J F Kincaid Smith P Becker G J Loughhead M G Freeman J W & Sands J M Goodpasture s syndrome in identical twins Ann Intern Med 88 62 1978
3 Glaeser J A Kleine Mitteilungen Z Klin Med 21 377 1892
4 Heale W F Matthiesson A M & Niall J F Lung haemorrhage and nephritis (Goodpasture s syndrome) Med J Aust 3 355 1969
5 Klavis G & Drommer W Goodpasture Syndrome und Benzineinwirkung Arch Toxicol 26 40 1970
6 Lagrue G Kamalodine T Guerrero J Hirbec G Zhepova F & Bernaudin J F Néphropathies glomerulaires primitives et inhalation de substances toxiques J Urol Nephrol 83 323 1977
7 Ravnskov U Exposure to organic solvents—a missing link in poststreptococcal glomerulonephritis? Acta Med Scand 203 351 1978
8 Ravnskov U Brun C Forsberg B Hedner U Jorgensen F Larsen S Laurell A B Sjoholm A & Skerfving S The features of glomerulonephritis in patients exposed to volatile hydrocarbons VII Int Congr Nephrol Montreal 1978
9 Ravnskov U Forsberg B & Skerfving S Glomerulonephritis and exposure to organic solvents A case-control study Acta Med Scand 205 575 1979
10 Seeliger K & Huland H Kasuistischer Beitrag zur Atiologie des Goodpasture Syndroms Med Klin 68 437 1973
11 Zimmerman S W Groehler K & Beirne G J Hydrocarbon exposure and chronic glomerulonephritis Lancet 2 199 1975

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Cellular Immunity in Renal Diseases

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ABSTRACT Cellular immune reactivity was studied in 78 patients with various forms of renal disease by skin testing with four recall antigens and a lymphocyte transformation test with tuberculin PPD and leucoagglutinin Patients with S-creatinine ≥230 μmol/l as well as those with chronic pyelonephritis who had S-creatinine values below 230 μmol/l had significantly lower skin reactions than the controls to streptokinase streptodornase, parotitis and PPD Glomerulonephritic patients with S-creatinine values below 230 μmol/l had normal skin reactivity Lymphocyte transformation tests showed decreased reactivity only in patients with S-creatinine level ≥230 μmol/l The results suggest an association of chronic pyelonephritis with a defective efferent, nonspecific arm of cellular immunity

Key words *pyelonephritis glomerulonephritis renal failure* cellular immunity

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Patients with uraemia may have depressed immunity Thus anergy to skin test antigens and reduced in vitro responses of lymphocytes to mitogens have been reported (3 5 7) It is claimed that uraemic toxins are responsible for these changes since uraemic serum and certain substances in renal failure inhibit blast transformation in vitro (9) The cause of renal failure may be of no consequence in this respect (7)

Yet even in the absence of uraemia patients with renal diseases may have lower cellular immunity This could play a role in the pathogenesis of the disease Thus in a study on urinary tract infections in children it was shown that skin test responses to PPD were weaker in patients with chronic and recurrent urinary tract infections and chronic pyelonephritis (2) The production of immune complex glomerulonephritis is easier when the animals used have an impaired cellular immunity (1) Nevertheless we found no information on cellular immunity in adult non uraemic patients with glomerulonephritis or pyelonephritis Therefore we decided to study skin reactions to various antigens as well as lymphocytic blast transformation in response to PPD and LA in patients with renal diseases both with and without renal insufficiency

PATIENTS

The following groups of subjects were studied (Table I)

Group *Ia* Twenty two patients had biopsy proven glomerulonephritis of varying types All had serum creatinine levels below 230 μmol/l

Group *Ib* Twenty two patients had chronic pyelonephritis The diagnosis was based in each case on two coexistent criteria a history of recurrent established urinary tract infection and demonstration of renal morphological alterations Twelve patients had calyceal deformities and corresponding cortical scarring in the i v pyelography Ten patients had renal biopsy changes compatible with chronic pyelonephritis Their serum *creatinine levels were below 230 μmol/l At the time of* examination only a few of these patients were being treated with ampicillin or sulphonamides but none with trimetoprim None used other drugs

Group II Thirty four patients had moderate or severe renal insufficiency (serum creatinine ≥ 230 μmol/l) Ten of them had biopsy proven glomerulonephritis Eight patients had chronic pyelonephritis defined by the presence of three diagnostic criteria a history of recurrent urinary tract infection renal morphological alterations and decreased renal function Seven patients had biopsy proven acute interstitial nephritis and nine miscellaneous renal diseases

Group III Twenty five patients who were hospitalized for various *non-renal disorders formed the control series* They were chosen to be age and sex matched with the patients of group *Ib*

Patients with malignancies infections or known immunological disorders were excluded None of the sub-

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Abbreviations PPD = tuberculin (pure protein derivative) LA leucoagglutinin SK SD = streptokinase streptodornase PAR = parotitis vaccine OM = oidiomycin

Table I *Number age and serum creatinine values (mean ± S D) of the groups studied*

	No of pats	Age (y) Median	Range	S creatinine (μmol/l)
Patients with S-creatinine <230 μmol/l	44	45	17–18	104± 37
Glomerulonephritis (*Ia*)	22	31	17–62	94± 30
Chronic pyelonephritis (*Ib*)	22	55	17–78	115± 42
Patients with S-creatinine ≥230 μmol/l (II)	34	52	17–79	664±479
Glomerulonephritis	10	54	30–67	817±672
Chronic pyelonephritis	8	58	42–79	637±407
Acute interstitial nephritis	7	23	17–46	721±346
Miscellaneous	9	52	31–71	474±359
Control patients (III)	25	49	15–74	86± 5

jects studied were treated with corticosteroids or immunosuppressive drugs All were in a satisfactory nutritional state

METHODS

Skin testing

Delayed type hypersensitivity reactions were examined in the patients and control subjects by skin testing with four different recall antigens tuberculin (PPD) (State Serum Institute Copenhagen Denmark) at 1 TU and 10 TU concentrations oidiomycin (OM) (Dermatophytin O Hollister Stier Laboratories Spokane Pa USA) at 1 5 or 1 50 dilutions of the stock solution streptokinase streptodornase (SK SD) (Lederle Laboratories N Y USA) at 5 IU and 50 IU concentrations of streptokinase and parotitis (PAR) (Parotitis vaccine from the State Public Health Laboratories Helsinki Finland) at 1 10 1 100 dilutions of the stock solution The test was rmed by injecting the four antigens at two con tions on the same occasion into both arms of the jects In each test 0 1 of the test antigen was injected intradermally

Reactions were read at 24 48 and 72 hours Induration and erythema larger than 5 mm Ø was taken to indicate a positive reaction

Lymphocyte transformation test

Lymphocyte responses to antigenic or mitogenic stimulation were measured by a transformation test using a micro whole blood method (4) Incorporation of 5 iodo-2-deoxyuridine was used to measure the DNA synthesis PPD was used as a specific antigen and leucoagglutinin (LA) as a nonspecific mitogen For the cultures heparinized venous blood (25 IU/ml preservative free heparin Medica Pharmaceutical Company Helsinki Finland) was drawn 1–2 hours before the initiation of the cultures Microtiter plates with U bottom 0 2 ml wells were used (Sterilin Middlesex UK)

Triplicate cultures of each concentration of antigen or mitogen were prepared as follows 0 1 ml of PPD at a final concentration of 12 5 and 50 μg/ml and LA at a final concentration of 12 5 and 25 μg/ml in MEM S (Orion Pharmaceuticals Helsinki Finland) were added to each well Control cultures contained no antigen or mitogen A drop of blood (0 025 ml) was added and the plates were incubated at 37°C in an atmosphere of 5% CO_2 in air for 72 hours for LA and 96 hours for PPD 18 hours before termination of the cultures 0 025 ml of the isotope solution containing 0 125 μCi of ^{125}I labelled 5 iodo-2-deoxyuridine (specific activity 90–100 mCi/mg Radiochemical Centre Amersham England) and a 10 $^{-3}$ molar solution of fluorodeoxyuridine (Sigma St Louis USA) in MEM S

Table II *Relative skin test reactivity (mean ± S D) and the percentage of negative skin test reactions with OM SK SD PAR and PPD*

	Relative skin test reactivity	Negative skin reactions (%)[c] OM	SK SD	PAR	PPD
Patients with S creatinine <230 μmol/l	0 82±0 47	57	23	31	19
Glomerulonephritis (*Ia*)	0 99±0 42	47	9	19	14
Chronic pyeloephritis (Ib)	*0 69±0 45*	67	38[b]	43[b]	24[a]
Patients with S-creatinine ≥230 μmol/l (II)	0 50±0 45[b]	73	47[b]	80[b]	33[b]
Control patients (III)	1 05±0 47	60	12	16	8

[a] $p<0\ 01$ [b] $p<0\ 001$
[c] No reaction with the highest antigen concentrations used

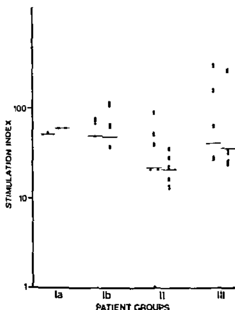


Fig 1 Lymphocyte stimulation indices with two concentrations of LA (▲=12 5 µg/ml and ●=25 µg/ml) in the groups studied Geometric mean is indicated by a horizontal line

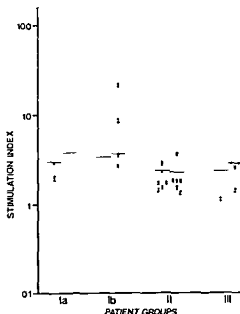


Fig 2 Lymphocyte stimulation indices with two concentrations of PPD (▲=12 5 µg/ml and ●=50 µg/ml) in the groups studied Geometric mean is indicated by a horizontal line

was added The cultures were terminated by harvesting on glass filters using a Scatron type harvester (Flow Laboratories Glasgow Scotland) and the filters counted in a gamma counter (Wallac/LKB Turku Finland) with an internal standard One minute counts were taken and the results were expressed as a stimulation index In this test the unstimulated values were usually in the range of 100 counts/min whereas the stimulated values were up to about 100 times higher (Figs 1 and 2)

To arrive at a standardized value for skin test reactivity the results obtained from the skin test in the control series were used as a reference as follows each antigen was given a score from 0 to 2 (0 = no reaction 1 = positive at the higher concentration 2 = positive at the lower concentration) Via calculation the mean score values for each antigen in the control series were 0 52 for OM 1 58 for SK SD 1 42 for PAR and 1 47 for PPD The relative index for each antigen and individual patient was then found from the formula

$$i_{OM} = \frac{\text{score OM}}{0\ 52} \qquad i_{SK\ SD} = \frac{\text{score SK SD}}{1\ 58}$$

$$i_{PAR} = \frac{\text{score PAR}}{1\ 42} \quad \text{and} \quad i_{PPD} = \frac{\text{score PPD}}{1\ 47}$$

A combined relative skin reactivity index was defined as

$$I = \frac{i_{OM} + i_{SK-SD} + i_{PAR} + i_{PPD}}{4}$$

Grouped samples were compared by Student s *t* test Results of negative skin reactions were evaluated by the χ^2 test

RESULTS

Results obtained in the skin testing are shown in Table II No differences were observed between various patient groups and control subjects when OM was used as test antigen perhaps because only 40% of the control population were positive to the highest antigen concentration The other three antigens (SK SD PAR and tuberculin PPD) produced significant differences in patients with S-creatinine ≥ 230 µmol/l versus controls both when results with individual antigens were evaluated and when relative skin test reactivity was used However in this group no significant differences were observed in the diagnosis of renal disease Patients with S creatinine < 230 µmol/l with chronic pyelonephritis showed a lower skin test reactivity with the three antigens compared with patients of the same group with glomerulonephritis or with controls The cases with low skin reactivity were scattered among the group The patients with normal serum creatinine values had equally impaired reactivity compared

with those in whom serum creatinine was slightly elevated

Results of lymphocyte stimulation are shown in Figs 1 and 2 Significant differences from control subjects were only seen in patients with S creatinine ≥230 μmol/l in cultures containing LA In the presence of PPD these patients gave a response that was equal to that of the controls

DISCUSSION

Depressed cellular immune response has been found in patients with renal insufficiency in several previous studies (5 7) While the reason for this is unclear the finding that some of the substances accumulating in the blood in uraemia are inhibitory to lymphocyte function may be of importance (9) Our results agree with previous findings since we noted a weak immune reaction in uraemic patients when they were examined by skin testing and by the lymphocyte stimulation test in the presence of LA

This study extended previous work by characterizing the immune status of patients with glomerulonephritis and pyelonephritis without renal insufficiency Normal immune reactions were observed in glomerulonephritic patients in fact the lymphocytic stimulation indices were higher in this group than in the control patients Therefore our results do not support the hypothesis that an altered r lower cellular immune response may contribute t the development of glomerulonephritis

Skin tests with a variety of recall antigens showed a low reactivity in our patients with chronic pyelonephritis Generally skin testing is not sufficiently accurate to measure immune responses since changes in reactivity can often be observed in consecutive tests carried out in the same subjects (8) Yet in our work the response to 3 out of 4 antigens used as well as the overall response measured with a relative skin reaction index was lower in patients with chronic pyelonephritis than in controls This supports previous results from studies on urinary tract infections and chronic pyelonephritis in children (2)

The lymphocyte stimulation indices with non specific mitogen and PPD were normal in the pyelonephritic patients As skin testing with PPD gave significantly lower values in the pyelonephritic group compared with controls a dissociation in the tests measuring different aspects of cellular immunity may exist as has been observed in other patient groups (6) The decreased skin reactivity in chronic pyelonephritis suggests a defective efferent nonspecific arm of cellular immunity The importance of this defect in the pathogenesis of chronic pyelonephritis remains unsettled

REFERENCES

1 Albini B Ossi E Brentjens J & Andres G Serum sickness glomerulonephritis in intact or thymectomized chickens Kidney Int 10 538 1976
2 Anttila R Grohn P & Krohn K Transfer factor and cellular immune response in urinary tract infections in children Acta Paediatr Scand 66 219 1977
3 Dobbelstein H Immune system in uraemia Nephron 14 409 1976
4 Eskola J Soppi E Viljanen M & Ruuskanen O A new micromethod for lymphocyte stimulation using whole blood Immunol Commun 4 297 1975
5 Huber H Pastner D Dittrich P & Braunsteiner H In vitro reactivity of human lymphocytes in uraemia—A comparison with the impairment of delayed hypersensitivity Clin Exp Immunol 5 75 1969
6 Kirkpatrick C Rich R & Smith T Effect of transfer factor on lymphocyte function in anergic patients J Clin Invest 51 2848 1972
7 Selroos O Pasternack A & Virolainen M Skin test sensitivity and antigen induced lymphocyte transformation in uraemia Clin Exp Immunol 14 365 1973
8 Thestrup-Pedersen K Temporary suppression of lymphocyte transformation after tuberculin skin testing Immunology 27 965 1974
9 Touraine J L Touraine F Revillard J P Brochier J & Traeger J T lymphocytes and serum inhibitors of cell mediated immunity in renal insufficiency Nephron 14 195 1975

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Identification of Renal Tubular Epithelial Cells in Urine with Immunofluorescence

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ABSTRACT Rabbits were immunized with human kidney homogenate The rabbit sera after absorption with human liver homogenate, showed antibody activity against human kidney, renal pelvis and urinary bladder The sera were then absorbed with human renal pelvis and urinary bladder mucosa and subsequently showed no antibody activity against urinary bladder or renal pelvis Immunofluorescence examinations showed fluorescing cells in kidney tubules, but not elsewhere The finally absorbed antihuman kidney sera were used for indirect immunofluorescence examination of urinary sediments from patients with renal disease Phase contrast microscopy was used simultaneously Fluorescence was found in cells which in phase contrast microscopy were judged to be renal tubular cells Fluorescing cells were often found in casts, but free cells were also seen Immunofluorescence may thus provide a means of identifying renal tubular cells in urine

Key words urine cytology phase contrast microscopy immunofluorescence kidney tubule

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Microscopic examination of urinary sediment can give valuable information about diseases in the kidneys or urinary tract (2 3 10 14 15 16 19 20 23 24 27 29) Depending upon the type of disease different types of cells may be found in the urine e g squamous epithelial cells urothelial cells renal tubular epithelial cells (REP) leukocytes and erythrocytes Proper identification of the cells is essential for obtaining optimal information (28) A correct identification of REP is especially important since an increased number of REP has been claimed to occur in certain diseases such as glomerulonephritis (3 10 24 28) and endemic benign nephropathy (29) or rejection of transplanted kidneys (2 20 23 25) In the opinion of most investigators REP can be identified with phase contrast microscopy (3 10 19 26) or various staining methods (2 15 20 21 23 25) The reliability of these methods for identification of REP in urine samples has however been questioned (9) The problems involved in the identification of REP have resulted in attempts to use special staining methods Thus Kaye (8) and Prescott and Brodie (17) advocated leukocyte staining methods in order to facilitate the differentiation of leukocytes from REP but the differentiation of REP from urothelial cells is a problem which remains unsolved with these methods Hardy (7) reported the application of the nitro blue tetrazolium (NBT) method for specific staining of REP in rat urine It should be pointed out however that also granulocytes will be stained by NBT at least in human blood (1) and human urine (own unpublished observations)

No staining method described hitherto seems to be specific for REP in urine The aim of the present study was to investigate whether an immunological method could be used for specific staining of REP while preserving the possibility of examination in phase contrast microscopy

MATERIAL AND METHODS

Human tissue material

Urinary bladder mucosa (Ubm) kidney pelvis mucosa (Kpm) and kidney parenchyma were obtained from two patients without anamnestic or histological evidence of kidney or urogenital disorders The preparations were

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Abbreviations REP = renal tubular epithelial cells NBT = nitro blue tetrazolium PBS = phosphate buffered saline ID = immunodiffusion IF = immunofluorescence Ubm = urinary bladder mucosa Kpm = kidney pelvis mucosa AHK = antihuman kidney serum AHKabsl = AHK absorbed with liver antigen AHKabslbp – AHKabsl absorbed with Ubm and Kpm antigen Ksup = kidney homogenate supernate Ksed = kidney homogenate sediment

recovered within 4–8 hours after death (from myocardial infarction and traffic accident respectively) Tissue blocks from the three tissues were kept frozen in isopentane at −70 C until analyzed

Preparation of tissue antigens

Kidney antigen The kidneys were freed from capsule and pelvic tissues Slices of cortex and medulla were homogenized for 10 min in a Sorwall omnimixer at 10 000 r p m under cooling in an ice bath with equal amounts (w/v) of 0 15 M phosphate buffered saline (PBS) pH 7 2 After centrifugation at 3 000×g the supernatant was saved (designated Ksup) and the sediment resuspended in 10 volumes of PBS and again homogenized for 3 min centrifuged at 5 000×g and the supernatant was discarded This procedure was repeated four times The final thoroughly washed sediment was resuspended in PBS in a volume giving the suspension an optical density of 1 0 at 502 nm in a 1 cm cuvette in a Vitatrone spectrophotometer and designated Ksed

Liver urinary bladder and kidney pelvis antigens Liver tissue from the same two patients was homogenized with equal amounts of PBS in a Sorwall omnimixer for 10 min as described above The whole homogenate was used in the absorption experiments while the supernate after centrifugation was used in the immunodiffusion (ID) analyses In a similar way homogenates and supernates were prepared from the Ubm and Kpm

Rabbit antihuman kidney serum

Rabbits were injected subcutaneously seven times at 2–3 week intervals with Ksed during a 3-month period The injections were given with 1 ml of Ksed the first two emulgated with complete and the last five with incomplete Freund's adjuvant (Difco) The rabbits were bled 2–3 weeks after the last injection The unabsorbed rabbit tihuman kidney serum was designated AHK

sorptions

bbit AHK was mixed with equal amounts of human liver tissue homogenate incubated at 37 C for 2 hours kept at 4 C over night and finally centrifuged at 5 000×g for 30 min The sediment was discarded and the supernate designated AHKabsl In the same way absorptions with Ubm and Kpm were performed and the absorbed sera were called AHKabslbp

Immunodiffusion (ID)

The antibody activities of the unabsorbed and absorbed sera were tested in a micromodification of the gel-chamber technique (30)

Immunofluorescence (IF)

IF analyses were performed using an FITC labelled antirabbit gammaglobulin serum from goat (Behringwerke lot 0102 P F/P ratio 4 0) A Leitz Orthoplane II microscope equipped with a Hg lamp and filters (BG 12 BG 38 K 470 and K 490) was employed For specificity testing of the rabbit AHK cryosections were prepared from renal pelvis urinary bladder and kidney containing cortex and medulla but no papilla Sections were incubated for 30 min at 22°C with AHK AHKabsl AHKabslbp in different dilutions and with preimmune rabbit sera respectively diluted 1/10 After washing with PBS sections were incubated with the FITC labelled goat antirabbit serum (see above) diluted 1/10 and washed

Urinary sediment

Urine was sampled from eight patients One patient had chronic pyelonephritis one secondary amyloidosis one diabetes mellitus with nephropathy two urinary tract infection two chronic glomerulonephritis and one Goodpasture s syndrome Urine from healthy individuals was not examined because the number of cells in urine from healthy individuals was considered to be too small for this kind of examination (28) Immediately after voiding the urine samples were centrifuged in three 10 ml plastic tubes One of the sediments was examined under a phase contrast microscope as described previously (7 26 27 29) After washing the sediment from the second tube was incubated with normal rabbit serum diluted 1/10 and the sediment from the third one with AHKabslbp After washing the last two sediments were incubated with FITC labelled goat antirabbit serum and washed twice Incubations were done for 30 min at 23 C in the dark Washings were done with 9 ml of PBS centrifugations at 1 000×g for 10 min and the sediments were resuspended between the different steps in the procedure The wet sediments were examined simultaneously with phase contrast microscopy and fluorescence (see above)

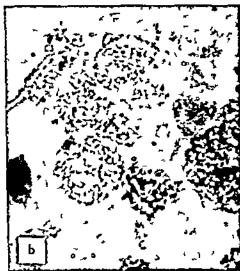
EXPERIMENTS AND RESULTS

Specificity controls of antisera

AHKabsl was tested in ID with Ksup and antigens prepared from liver urinary bladder and kidney pelvic tissues After the absorption with liver antibodies which reacted with antigens in the kidney kidney pelvis and bladder were still detected in AHKabsl The presence of antibodies in AHKabsl reacting with these tissues was also confirmed by the IF studies using sections from kidney liver kidney pelvis and urinary bladder Fluorescence was seen in all parts of the nephron

No fluorescence was seen in sections of Kpm or Ubm incubated with AHKabslbp while fluorescence was observed in the kidney tubules In some instances the fluorescence was clearly localized to the proximal tubules although it could not be stated unequivocally that all proximal tubules and only these were fluorescing The fluorescence was definitely strongest in the luminal part of the tubule cells probably representing the brush border region Fluorescence was never seen in glomeruli interstitial tissue or vessels Sections incubated with preimmune rabbit sera were constantly negative





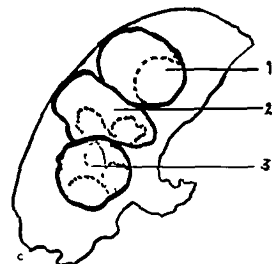


Fig 1 Urinary cast fragment containing three cells analyzed with immunofluorescence (*a*) phase contrast (*b*) and schematic drawing of the same field (*c*) The nucleus of a fluorescing cell is indicated (*1*) The size and form of the cell and its eccentrically located round nucleus are indicative of a tubular cell In phase contrast two other cells (*2* and *3*) are visible probably degenerated granulocytes The nuclear segments visible on the original photo are outlined ( ) The weak granular fluorescence located to cells 2 and 3 might be the result of phagocytosis of antigenic material Granulocytes outside casts were not fluorescing Magnification ×1000

Urinary sediment

Phase contrast examination of sediments incubated with sera did not reveal any differences e g in cell degeneration compared with unincubated controls Fluorescence microscopy examination of sediments incubated with AHKabslbp showed strong fluorescence in 20–50% of those cells which in simultaneous phase contrast microscopy were judged to be REP using criteria described earlier (10 26–29) The fluorescence was localized to the cytoplasm and/or the cell membrane (Figs 1 and 2) while nuclei were mostly dark Fluorescing cells were often found in casts (Figs 1 and 2) Fluorescence was also found in cells which were too degenerated to admit identification with phase contrast but the size and nuclear cytoplasmic ratio corresponded well to REP Sometimes a weak fluorescence was also seen in casts However after another washing with PBS the cast fluorescence disappeared while cells were still fluorescing Fluorescence was never seen in cells which could be identified as non REP Sediments incubated with preimmune rabbit sera were negative Sera from two rabbits immunized with two different materials were employed and showed identical reactions

DISCUSSION

Immune sera obtained through immunization of rabbits with human kidney antigen were found to



Fig 2 Cast fragment containing renal tubular cells The film was first exposed to ultraviolet light and then to phase contrast The bright areas represent tubular cells which due to the phase contrast exposure seem to be embedded in a granular non fluorescent cast matrix Magnification ×680

yield antibody activity against all parts of the nephron as well as Ubm and Kpm even after bsorption of AHK with liver antigen For that son absorptions of AHKabsl were performed ith Kpm and Ubm antigens After this absorption IF and ID examinations revealed antibody activity against a part of the kidney tubule probably the brush border region of the proximal tubule but no antibody activity against urothelial cells in the kidney pelvis urinary bladder or serum proteins

Antibodies to structural membrane antigens of the tubular epithelium in man and animals have been described previously by several authors (4 5 6 12) It has been demonstrated that human tubular antigen may cross react with antigens present in tissues outside the urinary tract (5 11) The presence of a similar cross reactivity e g with small intestinal epithelium was not checked in the thoroughly absorbed antisera employed in the present investigation as that seemed to be beyond its scope

Double layer IF technique used for examination of urinary sediments with AHKabslbp demonstrated the occurrence of fluorescing cells in patient urine most often in casts but free cells were also seen Their appearance corresponded well to generally accepted opinions about renal tubular cells in urine (10 15–21) We got the impression that the weak fluorescence sometimes seen in casts was unspecific because repeated washings made the cast fluorescence disappear without affecting the cell fluorescence Also the Tamm Horsfall protein does not occur in the proximal tubule (11) which was found to fluoresce in this study Thus it seems unlikely that the antibody activity should have been directed against Tamm Horsfall protein

In our view, this method has made it possible to demonstrate renal tubular cells in urine It is probable however, judging from the IF analyses of the cryosections with the absorbed immune sera that only a certain population of the tubular cells was recognized Another indication of the limitation of the method was the presence of IF negative cells with REP characteristics in the phase contrast microscopy examination of urinary sediments The specificity of the method for the urinary renal tubular cell population is however high as no cross reactions with other cells or antigens of the urinary tract or urine were observed This method provides a new approach to the study of urinary sediments The clinical significance of the findings of these IF positive tubular cells will be possible to assess after investigation of a larger patient material

REFERENCES

1 Bjorksten B The NBT test using venous and capillary blood Scand J Haematol 11 270 1973

2 Bossen E H Johnston W W Amatulli J & Rowlands D T Exfoliative cytopathologic studies in organ transplantation III The cytologic profile of urine during acute renal allograft rejection Acta Cytol 14 176 1970

3 Brody L H Salladay J R & Armbruster K Urinalysis and the urinary sediment Med Clin North Am 55 243 1971

4 Dinh B L Brassard A & Katiyar V N Experimental glomerulonephritis induced with the major antigen of rat kidney Int Arch Allergy 43 131 1972

5 Edgington T S Glassock R J & Dixon F J Autologous immune complex nephritis induced with renal tubular antigen I Identification and isolation of the pathogenetic antigen J Exp Med 127 555 1968

6 Edgington T S Glassock R J Watson J I & Dixon F J Characterization and isolation of specific renal tubular epithelial antigens J Immunol 99 119 1967

7 Hardy T L Identification of cells exfoliated from

the rat kidney in experimental nephrotoxicity Ann Rheum Dis 29 64 1970

8 Kaye M A peroxidase staining procedure for the identification of polymorphonuclear leukocytes and leukocyte casts in the urinary sediment N Eng J Med 258 1301 1958

9 Kern W H Epithelial cells in urine sediments Am J Clin Pathol 56 67 1971

10 Lindqvist B & Wahlin A Differential count of urinary leucocytes and renal epithelial cells by phase contrast microscopy Acta Med Scand 198 505 1975

11 McKenzie J K & McQueen E G Immunofluorescent localization of Tamm Horsfall mucoprotein in human kidney J Clin Pathol 22 334 1969

12 Miettinen A & Linder E Membrane antigens shared by renal proximal tubules and other epithelia associated with absorption and excretion Clin Exp Immunol 23 568 1976

13 Mondorf A W Kinne R Scherberich J E & Falkenberg F Isolierung enzymatische und immunologische Charakterisierung einer Plasma membranfraktion vom proximalen Tubulus der menschlichen Niere Verhandlungen der deutschen Gesellschaft für innere Medizin und deutsche Hamatologen 77 702 1971

14 Murphy G P Williams P D & Merrin C E Diagnostic value of lymphocyturia in renal allograft rejection in man Urology 2 227 1973

15 O Morchoe P J Riad W Cowles L T Dorsch R F & Frost J K Urinary cytological changes after radiotherapy of renal transplants Acta Cytol 20 132 1976

16 Papadimitriou M Chisholm G D Kulatilake A E & Shackman R Clinical evaluation of the urinary sediment after renal allotransplantation J Clin Pathol 23 99 1970

17 Prescott L F & Brodie D E A simple differential stain for the urinary sediment Lancet 2 940 1964

18 Rofe P The cells of normal human urine A quantitative and qualitative study using a new method of preparation J Clin Pathol 8 25 1955

19 Russo M A & Cocket A T K Microscopic urinalysis with phase contrast microscopy J Urol 107 843 1972

20 Schumann G B Burleson R L Henry J B & Jones D B Urinary cytodiagnosis of acute renal allograft rejection using the cytocentrifuge Am J Clin Pathol 67 134 1977

21 Scott J T Denman A M & Dorling J Renal irritation caused by salicylates Lancet 1 344 1963

22 Senator H Farbenanalytische Untersuchungen der Harnsedimente bei Nephritis Virchows Arch Pathol Anat 131 387 1893

23 Spencer E S & Posborg Petersen V The urinary sediment after renal transplantation Acta Med Scand 182 73 1967

24 Strauss H Uber Zytodiagnostik am Urin (Mit besonderer Berucksichtigung der Nephritiden) Folia Urol 9 313 1917

25 Taft P D & Flax M H Urinary cytology in renal transplantation Association of renal tubular cells and graft rejection Transplantation 4 194 1966

26 Wahlin A Differential count of urinary leukocytes and renal epithelial cells A comparison between phase contrast microscopy of unstained sediments and light microscopy of fixed and stained specimens Ups J Med Sci 82 43 1977

27 — The urinary sediment in hydronephrosis Acta Med Scand 201 449 1977

28 — Differential count and quantitative estimation of granulocytes mononuclear leukocytes and renal epithelial cells in urine Ups J Med Sci 83 109 1978

29 Wahlin A Lindqvist B & Nystrom K The urinary sediment in endemic benign nephropathy A phase contrast microscopy study Acta Med Scand 202 51 1977

30 Wadsworth C A microtechnique employing a gel chamber compared with other micro- and macroplate techniques for immune diffusion Int Arch Allergy Appl Immunol 21 131 1962



F g 2 Cast fragment contain ng renal tubular cells The f lm was f rst exposed to ultra olet light and then to phase contrast The bright areas represent tubular cells which due to the phase contrast exposure seem to be embedded n a granular non fluorescent cast matrix Magn f cation ×680

yield antibody activity against all parts of the nephron as well as Ubm and Kpm even after on of AHK w th liver antigen For that absorptions of AHKabsl were performed h Kpm and Ubm ant gens After th s absorption IF and ID exam nations revealed ant body activ ty against a part of the kidney tubule probably the brush border region of the prox mal tubule but no antibody activity aga nst urothelial cells in the kidney pelvis urinary bladder or serum prote ns

Ant bod es to structural membrane antigens of the tubular epithelium in man and animals have been described previously by several authors (4 5 6 1?) It has been demonstrated that human tubular ant gen may cross react with antigens present in tissues outs de the urinary tract (5 11) The pres ence of a similar cross reactivity e g w th small intest nal ep thel um was not checked in the thoroughly absorbed ant sera employed in the pres ent invest gation as that seemed to be beyond its scope

Double layer IF technique used for exam nation of urinary sediments with AHKabslbp demon strated the occurrence of fluorescing cells in pat ent urine most often in casts but free cells were also seen The r appearance corresponded well to gen erally accepted opinions about renal tubular cells n urine (10 15–?1) We got the impression that the weak fluorescence sometimes seen in casts was un spec fic because repeated washings made the cast fluorescence disappear without affecting the cell fluorescence Also the Tamm Horsfall prote n does not occur in the proximal tubule (11) which was found to fluoresce in this study Thus it seems unlikely that the antibody activity should have been d rected against Tamm Horsfall protein

In our view this method has made it possible to demonstrate renal tubular cells in urine It is prob able however judging from the IF analyses of the cryosections with the absorbed immune sera that only a certain population of the tubular cells was recognized Another ind cation of the l mitat on of the method was the presence of IF negative cells with REP characteristics in the phase contrast mi croscopy examination of urinary sed ments The specificity of the method for the urinary renal tubu lar cell population is however h gh as no cross reactions with other cells or antigens of the urinary tract or urine were observed Th s method provides a new approach to the study of urinary sediments The clinical s gn ficance of the findings of these IF pos tive tubular cells will be poss ble to assess after invest gat on of a larger pat ent material

REFERENCES

1 Bjorksten B The NBT test us ng venous and cap l lary blood Scand J Haematol 11 270 1973
2 Bossen E H Johns on W W Amatulli J & Row lands D T Exfol at ve cytopathologic stud es n organ transplantat on III The cytologic prof le of urine during acute renal allograft reject on Acta Cytol 14 176 1970
3 Brody L H Salladay J R & Armbruster K Urinalys s and the urinary sed ment Med Cl n North Am 55 ?43 1971
4 D nh B L Brassard A & Kaliyar V N Experi mental glomerulonephritis induced w th the major an t gen of rat k dney Int Arch Allergy 43 131 197?
5 Edg ngton T S Glassock R J & D xon F J Autologous mmune complex nephrit s nduced wi h renal tubular antigen I Ident f cat on and solat on of the pathogenet c ant gen J Exp Med 127 555 1968
6 Edgington T S Glassock R J Watson J I & Dixon F J Characterizat on and isolat on of spec f c renal tubular ep thel al ant gens J Immunol 99 119 1967
7 Hardy T L Iden f cat on of cells exfoliated from

Renal Function and Plasma Aldosterone during Acute Lithium Intoxication

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ABSTRACT Studies on plasma aldosterone, total serum protein, electrolytes, osmolal concentrations in serum and urine, creatinine, lithium and osmolal clearances were carried out on 14 patients suffering from lithium intoxication The determinations were done on samples obtained on admission and during the following twelve days of treatment with hemodialysis, sodium chloride loading, or forced diuresis Plasma aldosterone and total serum protein were increased, serum sodium concentrations and creatinine clearances were decreased on admission Serum osmolality was normal, urine osmolal con centration was just above that in plasma The results showed that the lithium intoxicated patients were moderately depleted of sodium and water on admis sion Plasma aldosterone, total serum protein and serum sodium were normalized during treatment Creatinine clearance increased but did not normalize in all patients Renal concentrating tests were per formed in five patients three to four weeks after lithium intoxication The renal concentrating ability was reduced in all The elevated plasma aldosterone seen in lithium intoxication might have been a result of sodium and water depletion It is suggested that impaired renal concentrating ability induced by lith ium may make some patients more susceptible to conditions which can lead to sodium and water loss, and thereby to lithium intoxication

Key words lithium intoxication plasma aldosterone renal function

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Lithium salts are frequently used as psychotropic drugs Lithium treatment is usually carried out without serious side effects but an unexpected rise in serum lithium concentration has been seen in patients on a constant dose of lithium In some patients lithium intoxication has occurred (6) The causes of lithium intoxication in patients on long term lithium treatment are still poorly understood Effects of lithium on kidney function might be in volved in the occurrence of gradually developing lithium intoxication since the main route of lithium excretion is in the urine (1 4) Lithium treatment can lead to a vasopressin resistant impairment in renal concentrating ability (3 5 11) lower the renal response to aldosterone (13) cause sodium deple tion and increase the level of aldosterone in plasma (8 14) The purpose of the present investigation was to study plasma aldosterone electrolytes in serum and urine renal function and renal con centrating ability in lithium intoxicated patients on admission and during the first days of recovery in order to determine which of these factors may play a role in the development and cure of lithium in toxication

PATIENTS

Seven women and seven men with a mean age of 51 years (range 29–65 years) were studied (Table I) The patients had been on lithium treatment for more than one year (1 3 to 12 years) and had been on a rather constant daily lithium dosage for months or years Previous studies sug gest that renal disease or loss of water and electrolytes can lead to decreased lithium elimination and thence to lithium intoxication (6) In this study the following conditions that might have contributed to lithium intoxication were fever associated with influenzal symptoms gastroenteritis bronchitis (nine patients) diuretics (two patients) anorexia and vomiting due to a peptic ulcer (one patient) acute pyelonephritis (one patient) acute mania with re duced fluid intake (one patient) Clinical symptoms of lithium intoxication had developed in the patients during two to eight days before admission (Table I) Mental and neurological symptoms characterized the slowly develop ing lithium intoxication while gastrointestinal symptoms were seen in only one patient (6) The initial symptoms were decreased alertness or slight apathy followed by muscular rigidity and some inconsiderable fasciculations with varying localization and slight ataxia (Grade I) The symptoms worsened gradually An impaired conscious ness more severe fasciculations and a coarse irregular

1/

Table I *Clinical data and treatment in 14 patients with lithium intoxication*

C_{Cr}=creatinine clearance SLi=serum lithium concentration HD–hemodialysis treatment FD–forced diuresis treatment SC sodium chloride treatment

Pat no	Age (y)	Sex	Duration of Li⁺ treatment (y)	Li dosage (mmol/d)	Duration of symptoms before treatment (d)	Grade of severity of symptoms	SLi at start of treatment (mmol/l)	C_{Cr} (ml/min) On admission	C_{Cr} (ml/min) At discharge	Treatment	Kidney biopsy
5	63	♀	10	24	8	III	3 15	17	25	HD	−
6	65	♂	7	48	8	III	2 90	45	85	HD	+
7	39	♂	3	48	4	III	1 80	12	130	HD	+
8	65	♂	10	32	4	III	2 50	20	50	HD	−
10	61	♂	4	48	2	III	2 75	20	44	HD	−
11	55	♀	6	24	6	III	4 50	21	85	HD	+
12	54	♀	6	32	2	II	2 50	30	58	HD	−
14	61	♀	3	48	4	III	2 50	27	63	HD	−
15	35	♀	8	40	2	II	3 45	78	114	FD+HD	+
16	39	♀	1 3	32	2	II	3 58	101	100	FD+HD	+
17	47	♂	12	32	4	II	2 47	42	40	SC	+
18	31	♀	4	32	2	II	1 75	85	80	SC	+
24	63	♂	5	16	8	III	2 05	4 5	45	HD	+
25	29	♂	4	48	4	II	2 52	51	56	FD+HD	−
Average	51		5 2	36	4		2 74	40	69		
Range	29–65	7/7	1 3–12	16–48	2–8		1 75–4 50	4 5–101	25–130		

tremor of the limbs and pronounced ataxia developed (*Grade II*) The severest state of lithium intoxication (Grade III) was characterized by a stupor like impairment of consciousness or coma vigil and spontaneous or latent twitching movements of the limbs

Twelve patients were treated with hemodialysis three of these (nos 15 16 and 25) received forced diuresis for e hours after admission but hemodialysis was started ause lithium clearance during this treatment did not exceed 25 ml/min (varying between 6 5 and 23 ml/min) and the patient s condition deteriorated Two patients (nos 7 and 18) were given sodium loading with isotonic sodium chloride 450 and 300 mmol respectively during the first 24 hours of treatment During this period lithium clearance of 18 and 32 ml/min respectively were obtained During the first 36 hours after admission patient no 17 received 650 mmol of isotonic sodium chloride Serum lithium concentration was reduced to 1 0 mmol/l during the period of

Table II *Studies on blood composition and kidney function in lithium intoxicated patients Values are mean ±S E M for 14 lithium intoxicated patients*

C_{Osm} = osmolal clearance C_{Cr} = creatinine clearance C_{Li} = lithium clearance

	Time after admission 3–6 hours	2–4 days	6–12 days
Plasma aldosterone (ng/100 ml)	34 8±4 7[a]	18 4+2 4[b]	13 2±2 0[b]
Serum sodium (mmol/l)	137±2 1	140+1 5	142±1 7[b]
Serum protein (g/100 ml)	7 04±0 18	6 71+0 14[b]	6 52±0 11[b]
Serum potassium (mmol/l)	3 48±0 20	3 29+0 11	3 30±0 10
Serum lithium (mmol/l)	2 74±0 20	0 57±0 04[b]	
Serum osmolality (mOsm/kg H_2O)	294+4 5	298±3 3	301±4 9
Urinary osmolality (mOsm/kg H_2O)	04+42	241±33	240+32
Urinary output (ml/min)	1 10±0 23	1 54±0 25	1 78±0 28
C_{Cr} (ml/min)	40±7 9	66±11[b]	69±8 1[b]
C_{Osm} (ml/min)	1 03±0 20	1 35+0 23	1 47±0 34
C_{Osm}/C_{Cr} (%)	2 86±0 48	2 04±0 36	2 19±0 30
C_{Li}	7 8±2 5	10 6±2 3[a]	
C_{Li}/C_{Cr} (%)	15 6±2 0	14 4±2 1	
Urinary sodium (mmol/h)	1 92±0 47	2 22±0 50	2 42±0 62

[a] Significantly different from normal values
[b] Significantly different from values obtained 3–6 hours after admission

Table III *Glomerular filtration rate effective renal plasma flow and results of renal concentration tests in 5 patients after renal function had stabilized more than 3 weeks after intoxication*

C_{125I} = [125]I iothalamate clearance C_{131I-h} = [131]I hippuran clearance S_{Osm} = osmolal concentration in serum U_{Osm} = osmolal concentration in urine C_{Osm} = renal osmolal clearance U = urine volume

	Renal clearances		Renal concentration test*					
Pat no	C_{125I} (ml/min)	C_{131I-h} (ml/min)	Urinary output (ml/min)	S_{Osm} (mOsm/kg H_2O)	U_{Osm} (mOsm/kg H_2O)	C_{Osm} (ml/min)	V–C_{Osm} (ml/min)	Serum sodium (mmol/l)
10	32	144	2 22	342	133	0 78	+1 22	170
11	86	385	0 33	298	414	0 92	−0 25	142
15	110	412	0 74	303	467	1 10	−0 39	144
16	136	510	0 33	303	408	0 48	−0 11	145
17	44	179	1 16	334	240	0 82	+0 32	151

All values obtained during the last period of water deprivation

treatment but a severe hypernatriemia developed because of lithium induced nephrogenic diabetes insipidus with a permanent positive free water clearance (Table III) (6) During hemodialysis an average lithium clearance of 95 ml/min was obtained and it is concluded that hemodialysis treatment is the most efficient way of removing lithium from the body (6) All patients recovered from the lithium intoxication within 30 days

PROCEDURE OF STUDY

Each patient was examined three times The first examination was carried out during the first three to six hours after admission before any treatment had been given Investigations were repeated two to four days after admission one to two days after treatment with sodium chloride or hemodialysis had been finished The last investigation was carried out six to twelve days after admission Clearance data were obtained during three to six hour periods at the first investigation and as 24-hour tests at the second and third investigations In five patients (nos 10 11 15 16 and 17) renal concentration tests were done three to four weeks after admission at a time where renal function had been stable for more than one week In four patients a 26-hour concentration test was carried out In patient no 10 the investigation had to be stopped after eight hours of water deprivation because of a rapidly increasing serum sodium and serum osmolal concentration

METHODS

Plasma aldosterone was measured with Meta Damkjær Nielsen s method (10) which is a radioimmunological measurement performed on plasma after previous extraction with dichloromethane purification on silicagel columns and chromatographic separation on paper The position of aldosterone on the paper was located by scanning Aldosterone antiserum was obtained from Research Plus Laboratories Denville New Jersey The three plasma aldosterone values for each patient were determined within the same analytical run Plasma aldosterone in healthy control subjects ranged from 3 7–25 7 ng/100 ml The coefficient of variation was 15%

Lithium in serum was determined by flame photometry (1) and in urine by atomic absorption (2) Sodium potassium creatinine and total protein in serum and urine were determined by a sequential multiple autoanalyzer

Osmolal concentration in serum and urine was determined by a 65 31 advanced osmometer

Renal osmolal clearance was calculated as urine volume (ml/min)×osmolal concentration in urine/osmolal concentration in serum Free water clearance was calculated as urine volume–renal osmolal clearance

For statistical calculations a Wilcoxon signed rank test was used

RESULTS

The results are given in Table II The concentration of aldosterone in plasma was elevated above normal levels on admission but decreased to normal levels after 2–12 days of treatment The concentration of sodium in serum was in the low normal range on admission but increased significantly after 6–12 days The total protein concentration in serum was increased on admission but decreased to normal levels after 6–12 days No changes of importance were found in serum potassium concentrations Serum osmolal concentrations did not change significantly during the course of the study In fact they remained within the normal range

Urinary output varied considerably during the period of treatment The osmolal concentration of urine was however low on admission After 2–4 days it had fallen further to below the osmolal concentration in serum

Creatinine clearance was significantly reduced on admission After 2–4 days of tre

at low normal levels Osmolal clearance did not invite attention during the study it was permanently below 2 0 ml/min The ratio between renal osmolal clearance and creatinine clearance did not change significantly during the study

Lithium clearance was reduced on admission and increased significantly after 2–4 days of treatment Fractional lithium excretion remained obviously below normal levels during the study (normal range 17–30%)

Sodium excretion in urine averaged 46 mmol/24 hours on admission and only a slight insignificant increase was seen during the study In all five patients (nos 10 11 15 16 and 17) in whom renal concentration tests were done a reduced concentrating ability was found Two patients (nos 10 and 17) produced a permanent hypo-osmolal urine in spite of increasing serum osmolal and serum sodium concentrations

DISCUSSION

In the majority of cases reported lithium intoxication has occurred during long term lithium treatment in patients on a constant daily dose of lithium which had not been changed for months or years (6) Since excretion of lithium in urine is the major factor determining the serum lithium concentration in patients on a constant dose of lithium an alteration in renal lithium excretion probably plays a role the rise in serum lithium concentration in lithium intoxicated patients Lithium intoxication is often preceded by events inducing water and electrolyte loss (e g fever anorexia gastrointestinal disorders with diarrhoea and/or vomiting and treatment with diuretics) (6) events which might alter renal lithium excretion Previously sodium depletion which also can affect renal lithium excretion has been described as a major factor in the development of lithium intoxication (12 14) Thus alterations in water and electrolyte metabolism may lead to a decrease in renal lithium excretion followed by a rise in serum lithium concentrations and lithium intoxication The elevated plasma aldosterone levels seen in patients suffering from lithium intoxication together with the slight decrease in their sodium concentration and the increase in the serum total protein concentration support the notion that a loss of water and sodium has taken place This observation is supported by the fact that the five patients studied all had impaired renal concentrating ability Depletion of sodium and water may have caused a decrease of glomerular filtration rate and thus contributed to the development of lithium intoxication

Lesions to the distal tubules occurring during lithium intoxication in dogs were described already by Radomski et al in 1950 (9) In eleven patients of our study renal function was markedly reduced on admission This reduction could be referred to fluid and salt depletion and/or to a toxic effect of lithium on the kidneys At discharge from hospital creatinine clearance on the average was in the lowest part of normal range and in seven of the patients it was permanently reduced Kidney biopsy was done in seven of the patients The results have been published previously (5 7) In the biopsy specimens no acute lesions were found but in patients on long term lithium treatment focal interstitial fibrosis and tubular atrophy was found There was a significant reverse correlation between the amount of atrophic tubules and the maximal renal concentrating ability This condition makes the patients susceptible to fluid and electrolyte loss On admission the patients of this study were depleted of water and sodium The elevated serum aldosterone might be a result of this volume and electrolyte depletion In spite of dehydration urine osmolal concentrations and osmolal clearances were low We suggest that lithium intoxication occurring in patients who have been on long term treatment may be caused by impaired renal concentrating ability probably due to a lithium induced chronic interstitial nephropathy

REFERENCES

1 Amdisen A Serum lithium determinations for clinical use Scand J Clin Lab Invest 20 104 1967
2 Amdisen A Serum level monitoring and clinical pharmacokinetics of lithium Clin Pharmacokinetics 2 73 1977
3 Forrest J N Jr Cohen A D Torretti J Himmelhoch J M & Epstein F H On the mechanism of lithium induced diabetes insipidus in man and rat J Clin Invest 53 115 1974
4 Gershon S & Yuwiler H Lithiumion A specific phychopharmacological approach to the treatment of mania J Neuropsychiat 1 229 1960
5 Hansen H E Hestbech J Amdisen A & Olsen S Renal function and renal pathology in patients with lithium induced impairment of the renal concentrating ability In Proc Eur Dial Transplant Assoc 14 518 1977
6 Hansen H E & Amdisen A Lithium intoxication

Report of 23 cases and review of 100 cases from the literature Q J Med 47 123 1978

7 Hestbech J Hansen H E Amdisen A & Olsen S Chronic renal lesions following long term treatment with lithium Kidney Int 12 205 1977

8 Pedersen E B Amdisen A Kirkegaard Hansen A & Darling S Plasma aldosterone during lithium treatment Neuropsychobiology 3 153 1977

9 Radomski J L Fuyat H N Nelson A A & Smith P K The toxic effects excretion and distribution of lithium chloride J Pharmacol Exp Ther 100 429 1950

10 Rask Madsen J Bruunsgaard A Munck O Nielsen M D & Worming H The significance of bile acids and aldosterone for the electrical hyperpolarization of human rectum in obese patients treated with intestinal bypass operation Scand J Gastroenterol 9 417 1974

11 Singer J Rothenberg E & Puschett J B Lithium induced nephrogenic diabetes insipidus In vivo and in vitro studies J Clin Invest 51 1081 1972

12 Thomsen K The effect of sodium chloride on kidney function in rats with lithium intoxication Acta Pharmacol Toxicol (Kbh) 33 92 1973

13 Thomsen K Jensen J & Olesen O V Effects of prolonged lithium ingestion on the response to mineralocorticoids in rats J Pharmacol Exp Ther 196 463 1976

14 Thomsen K Olesen O V Jensen J & Schou M The mechanism of gradually developing lithium intoxication in rats Current Developements in Psychopharmacology 3 155 1976

Mortality from Chronic Interstitial Nephritis and Phenacetin Consumption in Denmark

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ABSTRACT After 1950, a pronounced increase in the mortality from chronic interstitial nephritis among women was registered in Denmark This was suspected to be caused by a contemporary increase in the consumption of drugs containing phenacetin Restrictions were therefore imposed on the sale of such drugs in 1961 and consumption decreased sharply This report, based upon death certificates from 1941–76, shows that the mortality rate from chronic interstitial nephritis has decreased gradually among women in Denmark since 1960 and has now reached the same level in younger women as in the 1940 s

Key words interstitial nephritis phenacetin mortality rate chronic pyelonephritis

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In the nineteen fifties an increasing number of deaths caused by chronic pyelonephritis or chronic interstitial nephritis was registered in many western European countries This increase in the mortality rate from chronic interstitial nephritis was assumed to be caused to some extent by an increase in phenacetin consumption and precautions were taken to avoid abuse of phenacetin

In Denmark the mortality rate from chronic interstitial nephritis has increased since 1951 (4) General warnings and restrictions on sales of drugs containing phenacetin were made by the National Health Service in 1961

To support the hypothesis that the increase in mortality from renal disease in the 50 s was caused by chronic interstitial nephritis following a phenacetin abuse a fall in mortality from renal diseases should be expected as a consequence of the reduction in phenacetin consumption This study shows such a fall in mortality in women in Denmark Following the fall in consumption of phenacetin

MATERIAL

This report is based upon all Danish death certificates from 1941–76

In 1941 97% of all certificates were issued by a medical doctor Since 1950 more than 99% of all certificates have been issued by a physician

From 1941 to 1950 the diagnoses were coded according to the international classification ratified in Paris in 1937 Since 1951 diagnoses have been classified according to the WHO International Classification of Diseases and Causes of Death

Deaths from renal diseases are difficult to classify But despite considerable problems in distinguishing different renal diseases and even between the glomerulonephritis and pyelonephritis groups all diagnoses in the present study have been arranged under these two major groups (Tables I and II) Deaths caused by hypertrophy of the prostate (ICD 600–607) are not included in this study

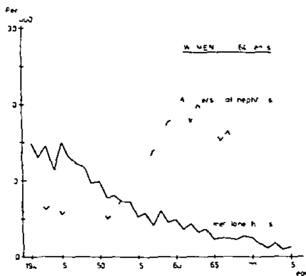
Estimation of the consumption of phenacetin during 1945–57 is based upon reports about the sale from 20 chemist s shops in different parts of the country (5) In 1960–67 the consumption is estimated by subtracting the export of phenacetin from the total Danish production and import Since 1972 the total sale of drugs from all chemist s shops has been reported annually to the National Health Service The sale of phenacetin during this period is based upon these reports

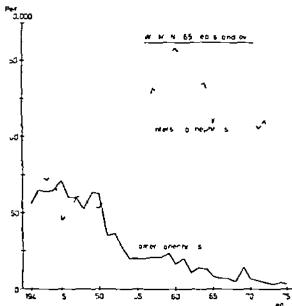
RESULTS

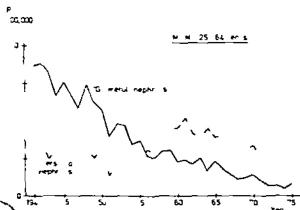
Since 1941 the death rate from glomerulonephritis has decreased gradually (Fig 1) In contrast to this

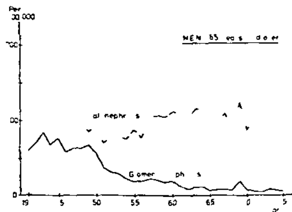
Table I *Renal diseases as cause of death 1941–50 (Paris classification)*

	1941	1945	1950
Nephritis acuta	18	47	15
Nephritis chronica	525	561	478
Glomerulonephritis total	543	608	493
Pyelitis et alii morbi renum et ureterum	294	197	294
Calculi viarum urinarum	115	130	139
Cystitis et alii morbi vesicae	37	21	16
Strictura et alii morbi urethrae	12	9	6
Chronic interstitial nephritis total	458	357	455









1 Mortality from glomerulonephritis and chronic interstitial nephritis in Denmark in 1941 1975

trend a remarkable increase in mortality from chronic interstitial nephritis among women occurred from 1950 to 1960 Thereafter the death rate decreased gradually and has now reached the same level as before 1950 for women below the age of 65

From 1945 to 1960 the consumption of phenacetin increased from about 4 to nearly 20 g per inhabitant per year In 1961 the consumption decreased sharply and has during the last ten years been about 4-5 g per inhabitant per year (Fig 2)

DISCUSSION

The evaluation of mortality rates by reviewing death certificates involves considerable problems This is partly due to changes in classification systems changes in diagnostic criteria and partly to lack of identical use of diagnostic criteria among physicians Variations over time in views about different diseases influence the choice of diagnosis

For example as the etiology is obscure in most cases of uremia in patients with small contracted

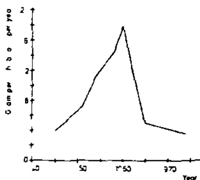


Fig 2 Consumption of phenacetin in Denmark in 1945-74

Table II *Renal diseases as cause of death 1951–75 (WHO classification)*

	1951	1955	1960	1965	1970	1975
Nephritis acuta	21	19	6	10	7	6
Nephrosis incl glomerulonephritis c nephrosis	25	43	24	17	5	2
Nephritis chron	248	181	187	95	88	51
Nephritis non spec	38	2	0	3	6	11
Alii nephropathiae chron	6	1	3	3	4	2
Glomerulonephritis total	338	246	220	128	110	72
Pyelitis pyelonephritis pyonephrosis abscessus renis	189	381	737	601	599	439
Hydronephrosis	14	8	7	6	5	4
Urolithiasis (calc renis et ureteris)	65	67	81	74	100	49
Alii morbi renis et ureteris	13	61	106	114	91	134
Calculus vesicae et urethrae	42	46	23	28	14	1
Cystitis	20	11	11	6	13	16
Alii morbi vesicae urinariae	6	2	6	7	3	3
Strictura urethrae	3	8	5	2	4	1
Alii morbi urethrae	–	2	3	2	–	–
Alii morbi tractus urinarii	–	–	–	–	5	7
Chronic interstitial nephritis total	352	586	979	840	834	654

kidneys physicians are liable to choose the most likely diagnosis A general view is that glomerulonephritis has become more rare and chronic pyelonephritis more common This leads to the choice of the latter diagnosis in cases of unknown etiology Thereby expectations can reinforce trends in mortality rates

However this phenomenon cannot explain the changes shown here in death rates from renal diseases as such changes in diagnostic habits would influence the choice of diagnosis in men and women equally

Mortality from renal diseases has been correlated to the consumption of phenacetin Since 1960 this has been reduced in Denmark from about 20 to about 4 g per inhabitant Furthermore abuse has practically upon closer scrutiny of the matter been eliminated It was characteristic for the pattern of consumption in the 50 s that some persons almost exclusively women had an intake of drugs containing phenacetin that was equivalent to an intake of 2–5 g phenacetin per day They took the analgetics for poorly defined reasons such as headache general fatigue etc When the abuse had been eliminated the average consumption had fallen to what must be considered a justifiable use of drugs containing phenacetin So far there is no evidence that this level causes renal damage

Harvald (3) showed that phenacetin abuse in Denmark was almost restricted to women In contrast to this the phenacetin abuse and increase in mortality from chronic interstitial nephritis in Huskvarna Sweden was almost restricted to men (1)

Trends in mortality rates from chronic interstitial nephritis seem to follow the consumption of phenacetin Such a correlation was first noticed in 1953 by Spuhler and Zollinger (16) Even though this does not prove any causal relationship there are heavy indications that an increase in death rate from chronic interstitial nephritis in the 50 s was caused by abuse of drugs containing phenacetin

REFERENCES

1 Bengtsson B & Hood B In Progress in pyelonephritis (eds E H Kass) p 297 Davis Co Philadelphia 1965
2 Causes of Deaths in the Kingdom of Denmark National Health Service of Denmark 1941–1975
3 Harvald B Am J Med 35 481 1963
4 Mosbech J Dan Med Bull 7 58 1960
5 Nissen N I Ugeskr Laeger 121 1055 1959
6 Spuhler O & Zollinger H U Z Klin Med 151 1 1953

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1α-hydroxyvitamin D_3 Treatment of Therapy-Resistant Symptomatic Hypocalcemia in a Hypoparathyroid Patient with Intestinal Malabsorption

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ABSTRACT The case history of a hypoparathyroid female with short bowel syndrome and long standing therapy resistant symptomatic hypocalcemia is reported During treatment with massive doses of the potent vitamin D analog 1α hydroxyvitamin D_3(1α(OH)D_3), normocalcemia was re established and clinical symptoms of hypocalcemia were relieved Furthermore, significant improvement of intestinal calcium absorption and bone mineral content was observed after three months of treatment with 1α(OH)D_3 The data suggest that 1α(OH)D_3 may be of therapeutical value in patients with hypoparathyroidism and intestinal malabsorption

Key words 1α(OH)D_3 hypoparathyroidism intestinal malabsorption

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In 1968 it was discovered (1) that vitamin D is hydroxylated in the liver at carbon atom 25 to form 25 hydroxyvitamin D_3 (25(OH)D_3) This metabolite has now been recognized as quantitatively the major circulating metabolite of vitamin D However in 1970 it was verified (4) that a very potent vitamin D metabolite 1 25-dihydroxyvitamin D_3 (1 25(OH)$_2$$D_3$) is produced exclusively in the kidneys Data obtained so far support the contention that 1 25(OH)$_2$$D_3$ is the biologically most active form of vitamin D (5)

Encouraging results have lately been reported from treatment of several clinical disorders with 1 25(OH)$_2$$D_3$ or with the analog 1α hydroxyvitamin D_3 1α(OH)D_3 (6) These disorders include renal osteodystrophy hypoparathyroidism vitamin D dependent rickets hypophosphatemic rickets and osteomalacia due to intestinal malabsorption (12) Realising that such a case has not previously been reported the present paper demonstrates the therapeutical effects of 1α(OH)D_3 in a patient suffering from hypoparathyroidism and intestinal malabsorption

CASE REPORT

A 78 year-old female was admitted in April 1978 The patient presented a 17 year history of severe symptomatic hypocalcemia Intestinal malabsorption of calcium and fat was present since 1961 when surgical complications following a gynecological laparotomy led to extensive resection of the small intestine Hypocalcemia and clinical symptoms of hypocalcemia deteriorated considerably following thyroidectomy which was performed in 1976 due to suspected cancer of the thyroid Histological examination of the removed gland showed Hashimoto s thyroiditis without signs of malignancy Thus presumably subtotal or total parathyroidectomy was accidentally done by this operation Despite treatment with large doses of oral calcium supplements (2–6 g/day) and oral (18 000–72 000 IU/day) or parenteral cholecalciferol (100 000 IU/month) *severe paresthesia in the hands and feet often associated* with carpopedal spasms had been present since the last operation

Before treatment with 1α(OH)D_3 (Leo Pharmaceutical Products) analyses showed serum ionized calcium (Ca^{++}) 0 65 mmol/l (normal range 0 99–1 15 (8)) serum total calcium 1 87 mmol/l (normal range 2 30–2 70) serum phosphate 1 78 mmol/l (normal range 0 80–1 48) serum magnesium 0.56 mmol/l (normal range 0 78–1 03) serum alkaline phosphatase 172 U/l (normal range 50–275) serum albumin 608 mmol/l (normal range 532–813) urinary calcium excretion 1 82 mmol/day and urinary phosphate excretion 5 65 mmol/day Fecal fat excretion amounted to 30–40 g/day despite treatment with a low fat diet (70 g/day) The fractional intestinal absorption of calcium (3) was 5 0% which is considerably below normal (normal range 27 1±7 0% (9)) Finally the bone mineral content (BMC) of the radius was low (0 476 g/cm) (normal range 0 788±0 232 (10)) (Fig 1)

When 1α(OH)D_3 treatment (5 μg/day orally) was initiated (Fig 1) Ca increased and clinical symptoms vanished after 14 days of treatment During careful control of Ca^{+} the dose of 1α(OH)D_3 was gradually increased to 12 μg/day During this regime normocalcemia was achieved within 3 weeks in spite of necessitated withdrawal of oral calcium supplements due to severe consti

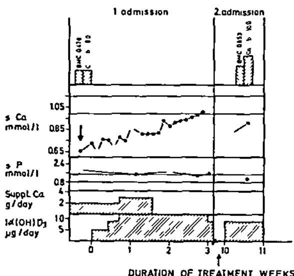


Fig 1 Results from $1\alpha(OH)D_3$ treatment of symptomatic hypocalcemia in a hypoparathyroid patient with intestinal malabsorption The arrows indicate presence of symptomatic hypocalcemia

pation Hyperphosphatemia diminished serum magnesium increased but remained subnormal (0 62 mmol/l) urinary calcium and phosphate excretion increased (Ca 5 09 mmol/day P 17 08 mmol/day) while fecal fat excretion was unchanged

The patient was discharged after 3 weeks of treatment ceiving a maintenance dose of 9 μg $1\alpha(OH)D_3$/day she ned normocalcemic and without clinical symptoms pocalcemia for the next 6 weeks Clinical and labora relapse occurred within 48 hours when the patient ccidentally discontinued the $1\alpha(OH)D_3$ treatment She was promptly readmitted to the hospital and $1\alpha(OH)D_3$ treatment was initiated again with almost immediate relief of clinical symptoms of hypocalcemia Upon re-estimation of intestinal calcium absorption and bone mass after 3 months of treatment with $1\alpha(OH)D_3$ it was disclosed that the intestinal calcium absorption had increased to 10 0% (still without any change in fecal fat excretion) and a significant increase in BMC (up to 0 653 g/cm) had taken place (Fig 1)

During further follow-up the patient has remained normocalcemic and without clinical symptoms of hypocalcemia for more than six months receiving $1\alpha(OH)D_3$ in a dose of 9 μg/day

DISCUSSION

Coincidence of hypoparathyroidism and severe intestinal malabsorption is rarely found Symptomatic hypocalcemia was the predominant clinical problem of the present patient Previous attempts to treat the condition with vitamin D_3 and/or oral calcium supplements had been unsuccesful Seen in the light of new discoveries in vitamin D metabolism this is not surprising It has been demonstrated that a partial block in renal 1α hydroxylation of vitamin D is present in surgical as well as idiopathic hypoparathyroidism (5) This is due to 1) lack of the stimulating effect on the biosynthesis of $1\ 25(OH)_2D_3$ which parathyroid hormone normally exerts 2) hyperphosphatemia frequently found in hypoparathyroid patients, which has been established as an inhibitor of the 1α hydroxylase of vitamin D Accordingly several groups have reported restoration of normocalcemia in hypoparathyroid patients treated with $1\alpha(OH)D_3$ or $1\ 25(OH)_2D_3$ (5 7 12)

Low levels of serum $25(OH)D_3$ have been found in patients with extensive resection of the small intestine (2) Apparently this is a reflection of malabsorption of vitamin D as well as loss of the enterohepatic circulation of the vitamin (2) Consequently intestinal malabsorption of calcium and thereby hypocalcemia in these patients is predominantly caused by reduced absorptive area of the small intestine and disturbed vitamin D metabolism

Thus the present patient suffered from several disorders leading to hypocalcemia It could be anticipated that a very large oral dose of metabolically active vitamin D was required to correct the condition

Possibly treatment with excessive doses of conventional vitamin D_3 orally or parenterally might have had the same good clinical effect However such a treatment would have been hazardous because of the risk of severe and long standing hypercalcemia The very short biological half life of $1\ 25(OH)_2D_3$ makes treatment with this compound or $1\alpha(OH)D_3$ far more safe Dihydrotachysterol is another vitamin D analog with a short biological half life Like $1\alpha(OH)D_3$ it does not require renal hydroxylation to exhibit physiological effect Dihydrotachysterol might well be beneficial to patients with total or partial block in the renal hydroxylation of vitamin D Comparative studies which at present are lacking should be carried out to determine the effects and side-effects of $1\alpha(OH)D_3$ and dihydrotachysterol during various clinical conditions

The tendency towards reduction of the increased serum phosphate in the present patient is probably a reflection of the effect on the renal tubule of in

creasing Ca^{++} (11) while the finding of an improved but still reduced intestinal absorption of calcium is a further demonstration of the very limited intestinal absorptive area available in this patient Finally it should be noted that even high doses of $1\alpha(OH)D_3$ were not sufficient to normalize serum magnesium It is possible that the patient would benefit from magnesium supplements

We conclude that large doses of $1\alpha(OH)D_3$ may be of considerable therapeutical value in patients with combined hypoparathyroidism and intestinal malabsorption

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REFERENCES

1 Blunt J W DeLuca H F & Schnoes H K Biochemistry 7 3317 1968
2 Compston J E & Creamer B Nutr Rev 35 297 1977
3 Curtis F K Fellows H & Rich C J Lab Clin Med 69 1036 1967
4 Fraser D R. & Kodicek E Nature 228 764 1970
5 Haussler M R & McCain T A N Engl J Med 297 974 1977
6 Holick M F Semmler E J Schnoes H K & DeLuca H F Science 180 190 1973
7 Jørgensen H & Vogt J H Acta Med Scand 201 3 1977
8 Madsen S & Ølgaard K Clin Chem 23 690 1977
9 — Eur J Clin Pharmacol 13 401 1978
10 Madsen S Ølgaard K & Ladefoged J Scand J Urol Nephrol 12 243 1978
11 Madsen S Ølgaard K & Thaysen J H Acta Med Scand 202 23 1977
12 Peacock M (ed) Clin Endocrinol vol 7 (Suppl) 1977

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Renal Function in Primary Hyperparathyroidism and in Non-Hyperparathyroid Hypercalcemia

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ABSTRACT Renal function was studied in 18 patients with moderate hypercalcemia Eight patients had primary hyperparathyroidism while 10 patients suffered from malignancies, vitamin D intoxication or sarcoidosis Glomerular filtration rate was significantly higher ($p<0\ 01$) in patients with primary hyperparathyroidism (mean serum calcium concentration 3 00 mmol/l) than in patients with hypercalcemia of non hyperparathyroid origin (mean serum calcium concentration 3 02 mmol/l) Renal plasma flow was 45% higher in hyperparathyroid patients than in non hyperparathyroid patients, but the difference was not significant Filtration fraction was higher ($p<0\ 05$) in patients with primary hyperparathyroidism than in other patients In seven patients with primary hyperparathyroidism, renal function was studied before and after parathyroidectomy In spite of a decrease in mean serum calcium concentration from 3 01 to 2 26 mmol/l, glomerular filtration rate and renal plasma flow remained unchanged In six patients with non hyperparathyroid hypercalcemia correction of the hypercalcemia (mean serum calcium concentration decreased from 2 99 to 2 34 mmol/l) was followed by significantly increased glomerular filtration rate ($p<0\ 01$) and renal plasma flow ($p<0\ 05$) Filtration fraction remained unchanged after correction of hypercalcemia in patients with primary hyperparathyroidism as well as in patients with non hyperparathyroid hypercalcemia These results suggest a different effect of calcium on the glomerular filtration rate and renal plasma flow in patients with primary hyperparathyroidism and in patients suffering from hypercalcemia of non hyperparathyroid origin These results also suggest that the presence of circulating parathyroid hormone in patients with primary hyperparathyroidism diminishes the depressive effect of calcium on the glomerular filtration rate

Key words hypercalcemia hyperparathyroidism renal function parathyroid hormone

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Hypercalcemia is a common electrolyte disturbance which is most often caused by cancer or hyperparathyroid disease (3) Hypercalcemia is less frequently associated with sarcoidosis and vitamin D intoxication (28) The kidney is particularly susceptible to hypercalcemia Impaired concentrating ability as well as decreases in renal blood flow and glomerular filtration rate (GFR) have been recorded in patients with hypercalcemic disorders (10) In a recently published retrospective study (17) serum calcium/serum creatinine and serum calcium/serum phosphate ratios were found to be significantly higher in patients with hypercalcemia caused by primary hyperparathyroidism than in patients with hypercalcemia of non hyperparathyroid origin This suggests a different effect of calcium on GFR in primary hyperparathyroidism and in non hyperparathyroid hypercalcemic disease In the latter case renal function is more sensitive to the influence of hypercalcemia

The aim of the present investigation was to study the effects of hypercalcemia on GFR and renal plasma flow in patients with hyperparathyroid hypercalcemia and patients with hypercalcemia of non hyperparathyroid origin In most patients renal function tests were repeated after treatment when the serum calcium levels had been normalised

PATIENTS

Renal function was studied in 18 hypercalcemic patients referred to the Department of Medicine for further investigation Thirteen patients were also studied after treat

Abbreviations GFR = glomerular filtration rate C_I = clearance of inulin C_{PAH} = clearance of para aminohippurate PAH = para aminohippurate FF = filtration tion C = creatinine clearance Ca [illegible] serum phosphate product (mmol/l

Table I *Age sex and histo pathological diagnoses in eight patients with primary hyperparathyroidism*

Pat no	Sex	Age (y)	Histo-pathological diagnosis
1	♂	68	Chief cell hyperplasia
2	♀	55	Chief cell adenoma
3	♂	30	Oxyphil adenoma
4	♀	70	Chief cell adenoma
5	♀	46	Chief cell adenoma
6	♂	62	Chief cell hyperplasia
7	♂	37	Chief cell hyperplasia
8	♂	59	Chief cell adenoma

ment Eight patients mean age 53 years had primary hyperparathyroidism Age sex and pathological diag noses are listed in Table I Parathyroidectomy was per formed in all patients but one patient number 2 who died of a myocardial infarction shortly before operation In ten patients mean age 54 years hypercalcemia was caused by a non hyperparathyroid disease (Table II) The carcinoma diagnoses in 3 patients were verified histo-pathologically The diagnosis of vitamin D intoxication in 2 patients was based on the case histories and the fact that the serum calcium concentration normalised when vitamin D medi cation was withdrawn In all patients with sarcoidosis a Kveim test using the membrane fraction of Kveim suspen sion (27) gave a positive reaction In 3 patients with dis seminated sarcoidosis patients number 14 17 and 18 the diagnosis was verified by biopsies from the liver and the spleen The non hyperparathyroid state in patients with hypercalcemia caused by malignancies or sarcoidosis was further verified by the finding of very low serum para thyroid hormone levels

METHODS

xperimental procedure

In all patients water diuresis was induced by drinking tap water 2% of the b w as a loading dose and 0 5% of the b w every 30 min throughout the study A standard clear ance technique was used

GFR was measured as the clearance of inulin (C_I) an renal plasma flow as the clearance of para aminohippura (C_{PAH}) both in ml/min and correlated to 1 73 m^2 surface After induction of water diuresis 0 5 ml/kg o a solution containing 85 mg/ml of inulin (Laevosa Gesellschaft) and 30 mg/ml of para aminohippurate (PAH (Merck Sharp & Dohme) was given as a loading dose The solution was then infused by a motor-driven syringe at a constant rate of 0 3 or 0 5 ml/min depending upon the renal function The equilibration time was 60 min Serum concentration of PAH did not exceed 190 μmol/l All patients were studied during three 20 min periods Mid period blood samples were drawn from an antecubital vein The bladder was catheterised and emptied at the end of each period by means of air insufflation and gentle suprapubic pressure

Analytical methods and calculations

Inulin in serum and urine was analysed by the method of Heyrovsky (12) and PAH by the method of Brun (4) Total calcium concentration in serum was measured by atomic absorption spectrophotometry (Perkin Elmer 403) Phosphate in serum was determined colorimetrically by the method of Fiske and Subbarow (11) Serum albumin was determined spectrophotometrically by the method of Doumas et al (8) Serum samples were analysed for im munoreactive parathyroid hormone by the method of Kleerekoper et al (15) An antiserum which reacts primarily with carboxyl determinants was used Filtration fraction (FF) was calculated as C_{In}/C_{PAH}

Standard statistical methods were used including paired observations and Student s *t* test All values are given as means ± S D

RESULTS

Renal function in hypercalcemia

In primary hyperparathyroidism mean serum cal cium concentration was 3 00±0 23 mmol/l in non hyperparathyroid hypercalcemia 3 02±0 27 mmol/l (Table III) GFR was 94±22 ml/min×1 73 m^2 in primary hyperparathyroidism and 47±33 ml/ min×1 73 m^2 in non hyperparathyroid hypercal

Table II *Age sex diagnoses and treatment in ten patients with hypercalcemia of non hyperparathyroid origin*

Pat no	Sex	Age (y)	Diagnosis	Treatment
9	♀	26	Carcinoma of the breast	Mastectomy fluoximesteron tamoxifen
10	♂	66	Carcinoma of the kidney	Corticosteroids medroxiprogesteron
11	♂	50	Carcinoma of the gall bladder	Doxorubicin fluracil lomustin
12	♀	51	Vitamin D intoxication	Withdrawal of vitamin D
13	♀	74	Vitamin D intoxication	Withdrawal of vitamin D
14	♂	29	Sarcoidosis	Corticosteroids splenectomy
15	♂	65	Sarcoidosis	Corticosteroids
16	♂	37	Sarcoidosis	Corticosteroids
17	♀	61	Sarcoidosis	Corticosteroids splenectomy
18	♂	78	Sarcoidosis	Corticosteroids

Table III *Serum calcium concentration serum phosphate concentration and renal function in eight patients with primary hyperparathyroidism and ten patients with non hyperparathyroid hypercalcemia*

	S Ca (mmol/l)	S P (mmol/l)	C_I (ml/min× 1 73 m²)	C_{PAH} (ml/min× 1 73 m²)	FF
Primary hyperparathyroidism (8 pats)					
Mean	3 00	0 67	94	426	0 23
S D	0 23	0 11	24	117	0 07
Non hyperparathyroid hyper calcemia (10 pats)					
Mean	3 02	1 19	47	294	0 17
S D	0 27	0 29	33	223	0 04
p	n s	<0 001	<0 01	n s	<0 05

cemia ($p<0$ 01) (Table III) Renal plasma flow was 45% *higher in hyperparathyroid patients than in* non hyperparathyroid patients but the difference was not significant (Table III) FF was higher in primary hyperparathyroidism ($p<0$ 05) Serum phosphate concentration was low 0 67±0 11 mmol/l in primary hyperparathyroidism and normal 1 19±0 29 mmol/l in non hyperparathyroid hypercalcemia ($p<0$ 001 Table III) Serum albumin was 40 5±4 7 g/l in primary hyperparathyroidism and 36 5±5 4 in non hyperparathyroid hypercalcemia ($p>0$ 05)

Renal function before and after parathyroidectomy

In seven patients renal function was studied before and 5 to 14 months (mean 8 3±3 5 months) after parathyroidectomy Mean serum calcium concentration decreased from 3 01±0 24 to 2 26±0 12 ($p<0$ 01) The individual changes are shown in Table IV GFR renal plasma flow and FF remained unchanged (Table IV) Serum phosphate concentration increased from 0 67±0 12 mmol/l to 0 91+0 19 mmol/l ($p<0$ 05)

Renal function before and after treatment of non hyperparathyroid hypercalcemia

In 6 patients with non hyperparathyroid hypercalcemia renal function was studied before and two weeks to three months (mean 2 3±2 3 months) after *starting treatment for hypercalcemia Mean serum* calcium concentration decreased from 2 99 to 2 34 mmol/l ($p<0$ 05) The individual changes show that GFR and renal plasma flow increased significantly while FF remained unchanged (Table V) Serum phosphate concentration was not altered by the treatment of the hypercalcemia

Table IV *Serum calcium concentration serum phosphate concentration and renal function before (B) and after (A) operation in seven patients with primary hyperparathyroidism*

Pat no	S-Ca (mmol/l)		S-P (mmol/l)		C_I (ml/min×1 73 m²)		C_{PAH} (ml/min×1 73 m)		FF	
	B	A	B	A	B	A	B	A	B	A
1	3 00	2 34	0 49	0 89	75	68	351	355	0 21	0 19
3	2 92	2 28	0 59	0 89	113	90	608	428	0 19	0 21
4	3 21	2 11	0 85	1 02	61	67	351	337	0 17	0 20
5	3 08	2 09	0 61	0 64	124	124	516	590	0 24	0 21
6	2 79	2 29	0 72	1 24	69	57	386	302	0 18	0 19
7	3 39	2 38	0 71	0 69	87	82	362	341	0 24	0 24
8	2 69	2 35	0 73	0 80	111	129	554	608	0 24	0 21
Mean	3 01	2 26	0 67	0 91	91	88	447	423	0 21	0 21
S D	0 24	0 12	0 12	0 19	25	28	109	126	0 03	0 02
p	<0 001		<0 05		n s		n s		n s	

p values are calculated by paired observations

Table V *Serum calcium concentration serum phosphate concentration and renal function before (B) and after (A) treatment in six patients with non hyperparathyroid hypercalcemic disease*

	S-Ca (mmol/l)		S-P (mmol/l)		C_{In} (ml/min×1 73 m)		C_{PAH} (ml/min×1 73 m²)		FF	
Pat no	B	A	B	A	B	A	B	A	B	A
9	3 11	2 52	1 72	1 35	118	145	810	900	0 15	0 16
13	3 30	1 71	1 44	1 45	29	50	112	250	0 26	0 20
15	2 80	2 56	1 16	0 65	58	80	413	574	0 14	0 14
16	2 83	2 41	0 89	0 77	23	31	184	167	0 13	0 18
17	3 29	2 41	1 09	1 06	59	68	358	391	0 16	0 17
18	2 61	2 45	1 04	1 01	41	62	278	371	0 15	0 17
Mean	2 99	2 34	1 22	1 05	55	73	359	442	0 17	0 17
S D	0 29	0 32	0 30	0 31	34	39	247	264	0 05	0 02
p*	<0 05		n s		<0 01		<0 05		n s	

* p values are calculated by paired observations

FF differed significantly between primary hyper parathyroidism and non hyperparathyroid hyper calcemic disease even after treatment of the hyper calcemia (p<0 01 Table IV Table V)

DISCUSSION

Impaired urine concentrating ability is usually seen in patients with hypercalcemia of varying origin (10) Hypercalcemia is also known to alter renal hemodynamics i e renal blood flow and glomeru lar filtration rate (17) In patients with primary hyperparathyroidism Mallette et al (21) found a ng negative correlation between serum calcium creatinine clearance (C ,) This inverse relation was also observed by Hodgkinson (13) who suggested that several factors could contribute to the renal damage e g hypercalcemia and hyper tension In another study Purnell et al (26) found glomerular filtration rate to be less than 60 ml/min in 8% of patients with proven hyperparathyroidism In a literature survey on hypercalcemia in malig nancies (1) renal function was reported to be im paired in all case reports containing notes about renal function Severe renal insufficiency in pa tients with hypercalcemia caused by malignancies has been reported by several authors (2 6 7 23 33) In a review of 160 cases of sarcoidosis Longcope and Freiman (18) found five instances of renal failure In four patients serum calcium was measured and high values found The relationship between impairment of renal function and hyper calcemia in sarcoidosis has also been demonstrated by Lofgren et al (20) The reversible renal insuffi ciency complicating hypercalcemia caused by sar coidosis has been described previously (24) Other studies indicate higher incidence of renal failure in patients with non hyperparathyroid hypercalcemia (sarcoidosis malignancies, vitamin D intoxication) than in patients with hypercalcemia caused by primary hyperparathyroidism Thus Transbol et al (31) found C_{cr} values below 50 ml/min in 63% of patients with non hyperparathyroid hypercalcemia but in only 18% of patients with primary hyper parathyroidism Similar results have been reported by Kistler and Neubauer (14)

GFR and renal blood flow have seldom been cor related with the serum calcium level However a previous study (17), showing higher serum cal cium/serum creatinine ratios in primary hyper parathyroidism than in hypercalcemia of non hyperparathyroid origin suggests a different effect of calcium on renal function in the two groups An analysis of data published by Transbol et al (30) shows that in patients with primary hyperpara thyroidism mean C_{cr} was 82 ml/min and in pa tients with non hyperparathyroid hypercalcemia 53 ml/min The serum calcium level was close to 3 1 mmol/l in both groups

In the present study in patients with hypercal cemia caused by primary hyperparathyroidism GFR was significantly higher than in patients with hypercalcemia caused by cancer vitamin D in toxication or sarcoidosis The mean age sex ratio and the serum calcium level were comparable in the two groups and could not explain the differences The renal plasma flow was 45% higher in hyper parathyroid patients than in patients with hypercal

li cemia of non hyperparathyroid origin but the difference was not statistically significant Libnoch et al (16) have recently published an interesting report on a patient with acute myelofibrosis malignant hypercalcemia and inappropriately high levels of parathyroid hormone In spite of a very high serum calcium level 5 63 mmol/l serum creatinine was only mildly elevated Woodhouse et al (32) have reported on four patients with thyrotoxicosis and hypercalcemia *In two patients high serum calcium* and urea levels were found Both responded to correction of the hyperthyroidism The hypercalcemia in the other patients was not associated with any rise in blood urea In these patients hyperparathyroidism was confirmed These clinical findings are in agreement with our results and indicate that high parathyroid hormone levels could modify the effects of hypercalcemia *on the renal function* Slatopolsky et al (29) have shown that purified bovine parathyroid hormone increased glomerular filtration rate It is possible that in hyperparathyroid hypercalcemia *circulating parathyroid hormone* acts in the same way modifying the negative effect of hypercalcemia on glomerular filtration rate and renal blood flow Higher serum phosphate concentrations *in non hyperparathyroid than in hyper* parathyroid patients at comparable serum calcium levels imply higher Ca×P products in non hyperparathyroid hypercalcemia than in primary hyperparathyr*oidism 3 7 and 2 1 respectively It can* not be ruled out that the higher Ca×P product in non hyperparathyroid hypercalcemia to some extent could explain the great influence of hypercalcemia on *renal hemodynamics in this group The* finding of a significantly higher FF in hyperparathyroid hypercalcemia suggests a relatively milder effect of calcium on GFR than on renal blood flow *in primary hyperparathyroidism com*pared to non hyperparathyroid hypercalcemia These findings are in agreement with the results of Brunette et al (5) They observed a reduction of FF in vitamin D *intoxicated dogs Brunette et al post*ulated that vitamin D intoxication decreases FF mainly by affecting the afferent arteriole

In seven patients studied before and after parathyroid*ectomy (Table IV) GFR renal plasma* flow and FF remained unchanged This in agreement with studies by Ohlsson (25) who performed C_i and C_{PAH} in 35 patients before and after parathyroid surgery *The clearances of inulin and* PAH were maintained both in subjects with normal and depressed clearance values before surgery Somewhat different results were obtained by Purnell et al (26) Preoperative and postoperative C_{cr} were available for 66 patients Nine patients who had significant impairment of glomerular filtration rate before operation had further deterioration in renal function after parathyroidectomy In six other patients there was an increase in GFR postoperatively and in the remaining 51 patients there was *no change In patients with hyperpara*thyroidism studied by Edvall (9) concentrating ability was generally improved after parathyroidectomy although C_{in} and C_{PAH} remained depressed The factors responsible for deterioration of renal function after successful removal of a parathyroid adenoma are not clear (21 26) Since parathyroid hormone has a vasodilating effect (22) the postoperative deterioration of renal function could be due to the fall in parathyroid hormone concentration following parathyroidectomy

In contrast to the patients with primary hyper*parathyroidism* patients with hypercalcemia of non hyperparathyroid origin showed reversible renal insufficiency (Table V) In all six patients studied before and after correction of hypercal*cemia C_{in} was improved regardless of treatment* Renal plasma flow increased in all but one patient FF remained unchanged Compared to the hyperparathyroid group (Table IV) FF was significantly *lower before as well as after correction of hypercal*cemia

The finding of reversible renal failure in non hyperparathyroid hypercalcemic patients and stable *renal function in hyperparathyroid patients in the* present study has been discussed earlier In a study by Lovice and Connor (19) C_{cr} was performed before and after correction of hypercalcemia Fol*lowing correction of hypercalcemia caused by* hyperparathyroidism C_{cr} remained normal in patients with initial normal values In five patients with reduced C_{cr} three showed improvement one *remained unchanged and two showed progressive* deterioration in renal function In the group with hypercalcemia of non hyperparathyroid origin a significant reduction in C_{cr} was found After treat*ment improvement in C_{cr}* was uniform with a mean rise of 20 ml/min following correction of hypercalcemia Kistler and Neubauer (14) studying renal function in patients with hypercalcemia caused by neoplasms found an increase in GFR in four out of five patients following treatment of hypercalcemia

In one patient GFR increased from 22 to 62 ml/min The increase in GFR following normalisation of serum calcium in non hyperparathyroid patients is probably due to a diminished vasoconstrictive effect of calcium but the normalisation of the Ca×P product could also play a part

The result that hyperparathyroid and non hyperparathyroid hypercalcemia have different effects on renal function is thus supported by results from previous studies Besides this difference found between two groups of patients with comparable age sex and level of hypercalcemia another difference concerning the effect of treatment on renal function reversible and non reversible renal failure respectively has been found The present results also confirm the results in the retrospective study regarding the effect of hypercalcemia on renal function The finding of reversible renal failure even in hyperparathyroid patients studied retrospectively suggests that the level of hypercalcemia also plays a part

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REFERENCES

1 Andersson Å & Bergdahl L Differentiation of acute hyperparathyroidism from severe hypercal[illegible] of malignancy Am J Surg 133 333 1977
2 Behn A R & West T F T Emergency treatment with calcitonin of hypercalcaemia associated with multiple myeloma Br Med J 1 755 1977
3 Benabe J E & Martinez Maldonado M Hypercalcemic nephropathy Arch Intern Med 138 777 1978
4 Brun C A rapid method for the determination of para aminohippuric acid in kidney function tests J Lab Clin Med 37 955 1951
5 Brunette M G Vary J & Carrière S Hyposthenuria in hypercalcemia A possible role of Intra renal Blood Flow (IRBF) Redistribution Pfluegers Arch 350 9 1974
6 Coombes R C Ward M K Greenberg P B Hillyard C J Tulloch B R Morrison R & Joplin G F Calcium metabolism in cancer Studies using calcium isotopes and immunoassays for parathyroid hormone and calcitonin Cancer 38 2111 1976
7 Cryer P E & Hill G J Pancreatic islet cell carcinoma with hypercalcemia and hypergastrinemia Cancer 38 2217 1976
8 Doumas B T Watson A W & Biggs H G Albumin standards and the measurement of serum albumin with bromcresol green Clin Chim Acta 31 87 1971
9 Edvall C A Renal function in hyperparathyroidism A clinical study of 30 cases with special reference to selective renal clearance and renal vein catheteriza tion Acta Chir Scand (Suppl) 229 1958
10 Epstein F H Calcium and the kidney Am J Med 45 700 1968
11 Fiske C H & Subbarow Y The colorimetric de termination of Phosphorus J Biol Chem 66 375 1925
12 Heyrovsky A A new method for the determination of inulin in plasma and urine Clin Chim Acta 1 470 1956
13 Hodgkinson A Biochemical aspects of primary hyperparathyroidism an analysis of 50 cases Clin Sci Mol Med 25 231 1963
14 Kistler H J & Neubauer W Phosphatbehandlung bei Hypercalcamie Klin Wochenschr 48 741 1970
15 Kleerekoper M Ingham J P McCarthy S W & Rosen S Parathyroid hormone assay in primary hyperparathyroidism Experiences with a radioimmunoassay based on commercially available reagents Clin Chem 20 369 1974
16 Libnoch J A Ajlouni K Millman W L Guansing A R & Theil G B Acute myelofibrosis and malignant hypercalcemia Am J Med 62 432 1977
17 Lins L E Reversible renal failure caused by hyper calcemia A retrospective study Acta Med Scand 203 309 1978
18 Longcope W T & Freiman D G A study of sar coidosis based on combined investigation of 160 cases including 30 autopsies from Johns Hopkins Hospital and Massachusetts General Hospital Medicine 31 1 1952
19 Lovice H & Connor T B Some observations on renal function before and after correction of hypercal cemia Ann Intern Med 58 744 1963
20 Lofgren S Snellman B & Lindgren Å G H Renal complications in sarcoidosis Functional and biopsy studies Acta Med Scand 159 295 1957
21 Mallette L E Bilezikian J P Heath D A & Aurbach G D Primary hyperparathyroidism clini cal and biochemical features Medicine 53 127 1974
22 Massry S G Coburn J W Friedler R M Kurokawa K & Singer F R Relationship between the kidney and parathyroid hormone Nephron 15 197 1975
23 Nelken R P Nieburg P I Bergstrom W H & Richman R A Dysgerminoma presenting as a cal cified abdominal mass with hypercalcemia Pediatrics 61 791 1978
24 Nilsson B S Hanngren Å Lins L E Ripe E Ivemark B Askergren J & Sundblad R Acute phase of sarcoidosis with splenomegaly and hypercal cemia Description of a case including a report about splenectomy and preparation and testing of a Kveim antigen from the spleen Scand J Respir Dis 59 199 1978
25 Ohlsson L Renal function in hyperparathyroidism A follow up study three to nine years after surgery comprising 35 cases Acta Endocrinol (Kbh) 63 161 1970
26 Purnell D C Smith L H Scholz D A El veback L R & Arnaud C D Primary hyper

parathyroidism a prospective clinical study Am J Med 50 670 1971
27 Ripe E Izumi T Kallner A Ljungquist A Nilsson B S & Unge G On the active principle in the Kveim suspension Scand J Respir Dis 54 111 1973
28 Singer F R Bethune J E & Massry S G Hypercalcemia and hypocalcemia Clin Nephrol 7 154 1977
29 Slatopolsky E Elkan I O Weerts C & Bricker N S Control of phosphate excretion in uremic man J Clin Invest 47 521 1968
30 Transbol I Hahnemann S & Hornum I The tubular reabsorption of calcium in primary hyperparathyroidism and in non-parathyroid hypercalcemia Acta Med Scand 184 33 1968
31 Transbol I Hornum I Hahnemann S Hasner E Öhlenschlager H Diemer H & Lockwood K Tubular reabsorption of calcium in the differential diagnosis of hypercalcemia Acta Med Scand 188 505 1970
32 Woodhouse N J Y Hoare A Mohamedally S M & Marsden P Thyrotoxicosis and hypercalcaemia Response to antithyroid drugs and salmon calcitonin Horm Res 7 238 1976
33 Zeffren J L & Heinemann H O Reversible defect in renal concentrating mechanism in patients with hypercalcemia Am J Med 33 54 1962

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Serum Calcitonin in Patients with Chronic Renal Disease

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ABSTRACT The relationship between serum calcitonin, calcium–phosphorus metabolism and renal function was studied in 66 patients with chronic renal disease Serum calcitonin was significantly elevated both in non dialysed patients and in patients on chronic hemodialysis In the non-dialysed patient group a highly significant inverse correlation was found between serum calcitonin and creatinine clearance Between serum concentrations of calcitonin and phosphorus a significant positive correlation was found In the dialysed patients a significant inverse correlation was found between serum calcium and serum calcitonin It is concluded that the elevated serum calcitonin in patients with chronic renal disease might be explained by a reduced renal degradation of calcitonin and/or an increased production due to stimulation by serum phosphorus

Key words serum calcitonin chronic renal disease serum calcium serum phosphate hemodialysis

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Increased levels of calcitonin have been reported in patients with chronic and acute renal failure (1 8 12) The reason for the elevated serum concentration may be a reduced renal elimination of the hormone or an increased production in patients with chronic renal failure (2 7 12)

Normal subjects (6) and patients with acute renal failure (1) have displayed positive correlations between serum calcitonin and serum phosphorus The increased level of serum calcitonin in chronic renal failure might therefore be explained by elevated serum phosphorus in this patient group (6 7) However no study has been made of the relationship between the degree of renal insufficiency and serum calcitonin or between serum phosphorus and serum calcitonin

The present investigation was therefore performed to elucidate the relationship between serum calcitonin and renal function and between serum calcitonin and serum concentrations of calcium and phosphorus in patients with chronic renal failure

PATIENTS

The study comprised 66 patients 32 men and 34 women with chronic renal disease Their mean age was 45 9 years (range 10–72) Creatinine clearance was 10–70 ml/min in 24 patients below 10 ml/min in 20 and below 2 ml/min in 22 patients the latter being on chronic hemodialysis None of the patients were treated with vitamin D metabolites The hemodialysed patients were dialysed for 6–9 hours weekly using a 1 m² 13 5 μm thick cuprophane membrane (Rhone-Poulenc RP 5) The dialysate contained 3 0 mEq/l of calcium

METHODS

Serum concentrations of calcium phosphorus creatinine protein and calcitonin were measured after an overnight fast Serum calcium was corrected for individual variation in serum protein concentration (11) and calculated as the calcium concentration corresponding to a protein level of 70 g/l (s calcium (corr)) Creatinine clearance was measured from 24-hour urines In hemodialysed patients the blood samples were taken before the start of dialysis The samples were centrifuged at 4 C after clotting and stored at −20°C until analysis

Calcitonin

Calcitonin was determined by radioimmunoassay using a commercial antibody to human calcitonin (Calbiochem USA) and synthetic human M-calcitonin (Ciba Switzerland) for standards and iodination ^{125}I calcitonin was prepared by the chloramin T method (13) The labelled antigen was purified by absorption to and elution from Quso G32 (Philadelphia Quartz Co Philadelphia USA) (13)

More than 90% of the labelled purified antigen could be bound by the antibody Assay conditions were modified from Dietrich et al (5) 200 μl serum and 250 μl antibody dilution in 0 1 M tris −0 25% HSA pH 7 3 was preincubated for two days at 4 C 50 μl ^{125}I-calcitonin dilution giving about 5000 cpm was added followed by two days incubation at 4 C Antibody bound calcitonin was precipitated by polyethylene glycol (PEG 6000) Standards were prepared in serum from total thyroidectomized patients Each serum was made in duplicate together with a tube

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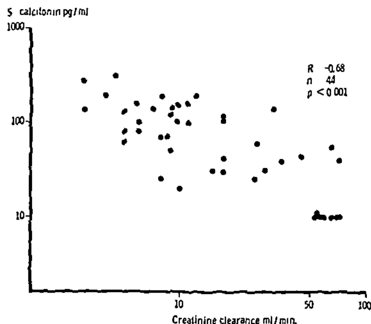


Fig 1 Relationship between serum calcitonin and creatinine clearance in non dialysed patients with chronic renal disease

without antibody to correct for incubation damage and non specific binding of the tracer The lowest detectable concentration was 20 pg/ml The precision of double determinations was 10 pg/ml Reproducibility was determined using a control serum with a calcitonin level of 140 pg/ml Over 9 assays the coefficient of variation was 14%

Statistical evaluations

rman s rank correlation test was used for correlation sis The Mann Whitney U test was used for com ns of group means

RESULTS

Non dialysed patients with chronic renal disease

Serum concentration of calcitonin was elevated to a mean value of 115 pg/ml (range 10–325) compared with a normal mean value of 38 pg/ml (range 10–120) ($p<0.01$) Fig 1 shows the relationship between creatinine clearance and serum calcitonin in the non dialysed patient group An increase in serum calcitonin with decreasing renal function was found ($R=-0.68$ $p<0.001$)

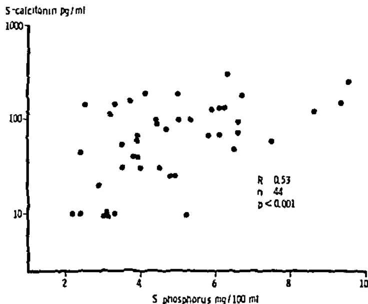


Fig 2 Relationship between serum calcitonin and serum phosphorus in non dialysed patients with chronic renal disease

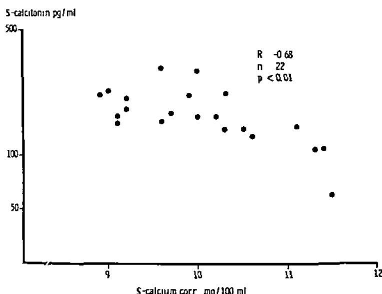


Fig 3 Relationship between serum calcitonin and serum calcium (corr) in patients on chronic hemodialysis

The relation between serum calcitonin and serum phosphorus is shown in Fig 2 A significant positive correlation was found between serum concentrations of calcitonin and phosphorus ($R=0.53$ $p<0.001$) Between the serum concentration of phosphorus and creatinine clearance there was a highly significant correlation ($R=-0.82$ $p<0.001$)

No significant correlation was found between serum calcitonin and serum concentrations of calcium ($R=0.02$ n s) or alkaline phosphatase ($R=0.16$ n s)

Patients on chronic hemodialysis

In patients on chronic hemodialysis the mean value of serum calcitonin was elevated to 178 pg/ml (range 60–310) Highly significant inverse correlations were found between serum calcitonin and serum calcium (total) ($R=-0.67$ $p<0.01$) and between serum calcitonin and serum calcium (corr) ($R=-0.68$ $p<0.01$) (Fig 3) Between serum calcitonin and serum concentrations of phosphorus ($R=-0.33$ n s) and alkaline phosphatase ($R=-0.12$ n s) no significant correlation could be demonstrated in this patient group

DISCUSSION

In agreement with previous studies (8 12) the present investigation shows increased serum concentrations of calcitonin in patients with chronic renal failure compared with normal controls Further a highly significant correlation was found between creatinine clearance and serum calcitonin Between the serum concentrations of phosphate and calcitonin there was a significant positive correlation whereas no significant correlation was seen between serum calcium and calcitonin

This finding might be a consequence of an increase in both serum phosphorus and serum calcitonin caused by decreased renal elimination in patients with chronic renal failure as a highly significant correlation was found between serum phosphorus and creatinine clearance However a significant correlation between serum concentrations of calcitonin and phosphorus has been found both in a group of normal subjects and in a single person during phosphate infusion (6) In an investigation of calcitonin in patients with acute renal failure (1) significant positive correlations were found between serum concentrations of phosphorus and calcitonin in 4 of 11 patients but not in the group as a whole

An increase in the calcium concentration in serum induced by calcium infusion has been shown to stimulate the secretion of calcitonin in normal subjects (6) but not in patients on chronic hemodialysis (9) On the other hand i v calcitonin administration may decrease the serum concentration of calcium and phosphorus in hemodialysis patients whereas no effect has been shown in normal controls (4)

In the present study no correlation was found between serum calcium (corr) and serum calcitonin in non dialyzed patients In the hemodialysed patients however we demonstrated a significant inverse correlation This finding agrees with that of Silva et al (12) in a study of 17 patients on chronic hemodialysis and might be explained by a suppression of serum calcium secondary to the hypercalcitonemia The lack of a relation between s calcium (corr) and serum calcitonin in non dialysed patients indicates that a calcium induced increase in serum calcitonin plays only a minor role in patients with chronic renal failure

Gastrin induced stimulation of calcitonin secretion has been proposed as a possible reason for the increased serum calcitonin in patients with chronic renal failure (1 12) It is well known that i v pentagastrin leads to a marked increase in serum calcitonin in normal subjects and in patients with medullary thyroid cancer (6 10) Increased serum gastrin concentrations have been found in patients with chronic renal failure (3 12) However a low but significant correlation was found between the serum concentrations of these hormones in a study of the interrelationship between serum gastrin and serum calcitonin in patients with chronic renal failure (3)

n conclusion the present study shows a close ion between the serum concentration of itonin and the renal function in patients with ronic renal failure The correlation between the serum concentrations of calcitonin and phosphorus suggests that increased serum phosphorus may act as a stimulator in the calcitonin production in these patients

ACKNOWLEDGEMENT

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REFERENCES

1 Ardaillou R Beaufils M Nivez M P Isaac R Mayaud C & Sraer J D Increased plasma calcitonin in early acute renal failure Clin Sci 49 301 1975
2 Ardaillou R Sizonenko P Meyrier A Vallee G & Beaugas C Metabolic clearance rate of radioiodinated human calcitonin in man J Clin Invest 49 2345 1970
3 Christensen C K Nielsen H E Kamstrup O Olsen K J Brandsborg O & Brandsborg M Increased serum gastrin and serum calcitonin in patients with chronic renal failure Acta Endocrinol (Kbh) In press 1979
4 Cochran M Hillyard C J Dew G J & Martin T J Acute responsiveness to calcitonin in chronic renal failure Br Med J 2 396 1976
5 Dietrich F M Hunziker W H & Fischer J A Synthetic human calcitonin Analysis of antibodies obtained from various animal species and determination of immunoreactive hormone in human sera Acta Endocrinol 80 465 1975
6 Franchimont P & Heynen G Parathormone and calcitonin radioimmunoassay in various medical and osteoarticular disorders Masson Paris 1976
7 Heynen G & Franchimont P Human calcitonin and serum phosphate Lancet 1 627 1974
8 — Human calcitonin radioimmunoassay in normal and pathological conditions Eur J Clin Invest 4 213 1974
9 Isaac R Nivez M P Piamba G Fillastre J P & Ardaillou R Influence of calcium infusion on calcitonin and parathyroid hormone concentrations in normal and hemodialyzed subjects Clin Nephrol 3 14 1975
10 Milhaud G Ribeiro F M Calmettes G Taboulet J Coutris G & Moukhtar M S Épreuves de stimulation de la secretion de calcitonine Intéret dans les cancers médullaires de la thyroide Nouv Presse Med 4 1793 1975
11 Pedersen K O Protein bound calcium in human serum Quantitative examination of binding and its variables by a molecular binding model and clinical chemical implications for measurement of ionized calcium Scand J Clin Lab Invest 30 321 1972
12 Silva O L Becker K L Shalhoub R J Snider R H Bivins L E & Moore C F Calcitonin levels in chronic renal disease Nephron 19 12 1977
13 Tashjian A H Jr Immunoassay of thyrocalcitonin I The method and its serological specificity Endocrinology 84 140 1969

Serum Calcitonin after Renal Transplantation

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ABSTRACT A prospective study of serum calcitonin was made in 9 patients with chronic renal failure The patients received a well functioning renal transplant At the time of transplantation serum calcitonin was significantly elevated as compared with the serum concentration in normal controls During the first 3–5 weeks after renal transplantation serum calcitonin decreased Afterwards it increased to a level of the same magnitude as the initial value A significant relationship was found between serum calcitonin and serum phosphorus, whereas no significant correlation was present between serum calcitonin and serum creatinine or between serum calcitonin and serum calcium

Key words renal transplantation serum calcitonin serum calcium serum phosphorus

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Increased serum calcitonin (s CT) concentrations are found both in patients with acute (1) and chronic renal failure (12) This has been attributed both to a decreased renal elimination of calcitonin (CT) and an increased CT secretion due to hyperphosphatemia (6 12)

S CT after renal transplantation (RT) has only been sporadically investigated Silva et al (12) found normal s CT concentration in 8 out of 11 patients and elevated s-CT in 3 patients with functioning kidney transplant during at least one month However changes in s CT after RT were not described in this study

The present investigation is a prospective study carried out to elucidate changes in s-CT concentration and interrelationships between s-CT and s-calcium s phosphorus and renal function after RT

PATIENTS

The study comprised 9 patients 3 males and 6 females 20–58 years old (mean 40 7) All the patients received a cadaver kidney and obtained good renal transplant function with creatinine clearance (Ccr) of 45–60 ml/min Further clinical data are given in Table I

Immunosuppressive therapy with prednisone and azathioprine was given in every case The initial daily dose of prednisone was 2 mg/kg b wt During the first month this steroid dose was reduced to about 30 mg/day

The usual daily dose of azathioprine was 2 mg/kg b wt Aluminium aminoacetate was given as antacid in a dose of 1 6 g/day for a mean of 23 days (range 17–40)

METHODS

Serum concentrations of calcium (corrected for individual variation in protein binding) inorganic phosphate creatinine and calcitonin were measured after an over night fast every week during the initial stay in hospital after RT as described in our previous paper (16) Furthermore studies of the specificity of the CT antibody was carried out No cross reactivity was found with synthetic human gastrin I (Imperial Chemical Industries Ltd Cheshire England) in a range of 100–1 000 pg/ml highly purified bovine parathyroid hormone (Wilson Laboratories Chicago Ill USA) in a range of 100–4 000 pg/ml highly purified porcine cholecystokinin and synthetic cholecystokinin like octapeptide (SQ 19 844) (provided by professor J F Rehfeld Århus Denmark) in ranges of 250–2 000 pg/ml synthetic cyclic human somatostatin (supplied by dr S Engkjær Christensen Århus Denmark) in range of 125–1 000 pg/ml or porcine monocomponent insulin (Novo Denmark) in a range of 100–4 000 μU/ml No interference with creatinine (0 03–0 20 mg/ml) urea (0 9–3 4 mg/ml) human albumin (2 5–100 mg/ml) or sodium chloride (120–150 mEq/l) was found in the range examined

Statistic evaluations were done as described in our paper in this issue (16)

RESULTS

S-CT was elevated to a mean value of 149 pg/ml (range 80–210) at the time of RT compared with a normal mean value of 38 pg/ml (range 10–120 $n=38$) ($p<0\ 01$) During the first 3–5 weeks after RT s CT decreased (Table II) During the following weeks there was observed an increase to a serum

Abbreviations CT = calcitonin RT = [...]tion S-CT = serum calcitonin Ccr =

Table I *Clinical data on the 9 RT patients*

CGN = chronic glomerulonephritis U = unknown CPN = chronic pyelonephritis AN = analgesic nephropathy CRD = congenital renal disease

Pat no	Age (y)	Sex	Renal disease	Duration of dialysis (mo)	Aluminium amino-acetate treatment (days after RT)
1	58	♀	CGN	6	2–26
2	51	♀	U	15	3–20
3	40	♀	CPN	0	2–18
4	57	♀	AN	1	3–41
5	47	♂	CGN	13	3–23
6	40	♀	U	11	2–19
7	20	♀	CRD	19	2–19
8	25	♂	U	14	2–26
9	28	♂	CPN	6	3–22
Mean	40 7			9 4	2–24

concentration of the same magnitude as the initial value In some patients a second decrease appeared thereafter

Fig 1 shows the mean values (±S E M) of CT phosphorus calcium corr and creatinine before and during the first 6 weeks after RT A significant correlation between the mean values of s CT and s phosphorus was found ($R=0\ 88$ $p<0\ 05$) during the first 6 weeks after the RT whereas no significant relation was seen between the mean values of s CT and s calcium corr ($R=0\ 61$) nor between T and s creatinine ($R=0\ 62$) s CT and Ccr −0 68) or between s phosphorus and Ccr −−0 78 $n=7$ $0\ 10>p>0\ 05$) No relation between changes in s CT and in the dosage of steroid was found

DISCUSSION

In agreement with previous studies of s CT in patients with uremia (12) increased s CT was found at the time of RT Improved renal function was accompanied by a decrease in s CT and later by an increase in s CT to a serum concentration of the same magnitude as the initial value

The high degree of correlation between s phosphorus and s CT in patients who obtained a well functioning renal transplant could support the suggestion that s CT plays a role in the regulation of s phosphate (6) As s phosphorus will decrease with improved renal function the relation between s CT and s phosphorus might partly be explained by the normalization of renal function after RT

In accordance with this we have previously found

Table II *S CT (pg/ml) before and after RT in 9 patients with a well functioning renal transplant*

Pat no	Before RT	After RT (weeks) 1	2	3	4	5	6	7	8	9
1	175	145	140	70	75	125	145	150	10	–
2	160	–	150	135	95	90	20	55	–	60
3	110	–	70	10	40	180	280	–	–	–
4	80	85	35	10	20	60	60	10	35	40
5	200	165	75	–	90	140	200	140	–	100
6	125	220	–	185	125	70	90	105	135	135
7	130	70	85	75	75	95	140	–	–	–
8	150	145	170	100	60	80	100	115	160	170
9	110	85	70	65	75	–	[illegible]	55	70	–
Mean	138	131	99	81	73	105	131	90	82	101
n	9	7	8	8	9	8	9	7	5	5
*p**	–	n s	<0 05	n s	=0 01	n s	n s	=0 02	–	–

* Mean s CT compared with the serum concentration before RT using the Wilcoxon test for paired differences

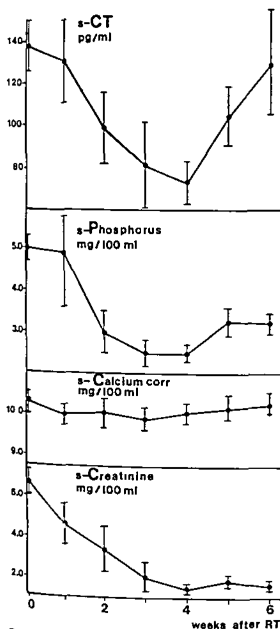


Fig 1 Serum concentrations of CT phosphorus calcium corrected and creatinine (mean±S E M) before RT and during the first 6 weeks after RT in 9 patients who obtained good kidney function

a close relationship between s CT and Ccr in non dialyzed patients with chronic renal failure (16)

The increase in s CT 3–5 weeks after RT was however not induced by changes in renal function or in the steroid dosage but coincided with the withdrawal of aluminium aminoacetate

A significant relation between s CT and s phosphorus has previously been demonstrated both in patients with acute (1) and chronic (12) renal failure and in normals (6)

The significance of the changes in s CT after RT is not clear However it seems that the decrease in s CT during the first weeks after RT may be caused by the normalization of renal function combined with an aluminium aminoacetate induced decrease in s phosphorus to subnormal serum concentrations Hypophosphatemic osteomalacia has been described both in patients on chronic hemodialysis (17) and in renal transplant patients (15) As the dosage of aluminium aminoacetate given before RT as phosphate binder and after RT as antacid experimentally may induce hypophosphatemic osteomalacia (18) the hypophosphatemia seen in the first weeks after RT may be explained by the treatment with aluminium aminoacetate A decrease in CT secondary to the hypophosphatemia might be a contributory factor in the development of bone lesions following RT as CT inhibits bone resorption In accordance with this Heynen et al (14) suggested that endogenous CT protects against uremic bone lesions in chronic hemodialysis patients

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REFERENCES

1–13 See our previous paper in this issue
14 Heynen G Kanis J A Oliver D Ledingham J G G & Russel R G G Evidence that endogenous calcitonin protects against renal bone disease Lancet 2 1322 1976
15 Moorhead J F Wills M R Ahmed K Y Baillod R A Varghese Z & Tatler G V Hypophosphatemic osteomalacia after cadaveric renal transplantation Lancet 2 694 1974
16 Nielsen H E Christensen C K & Olsen K J Serum calcitonin in patients with chronic renal disease Acta Med Scand 205 619 1979
17 Pierides A M Ellis H A Simpson W Dewar J H Ward M K & Kerr D N S Variable response to long term 1α hydroxycholecalciferol in haemodialysis osteodystrophy Lancet 1 1092 1976
18 Stanbury S W Azotaemic renal osteodystrophy Clin Endocrinol Metab 1 267 1972

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Serum Levels of Testosterone and Luteinizing Hormone in Patients with Chronic Renal Disease

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ABSTRACT Androgen metabolism has been studied in 33 patients (17 males, 16 females) with chronic renal disease not undergoing dialysis treatment The mean value of serum testosterone was reduced in both sexes, whereas that of serum luteinizing hormone (LH) was elevated in the males The parameters became increasingly pathological with decreasing renal function There was no correlation between serum testosterone and serum LH indicating an inadequate hypothalamic pituitary response to the testicular dysfunction The clinical significance of this relative hypoandrogenaemia is obscure A possible relation to the anaemia and bone disease of chronic renal failure is discussed

Key words chronic kidney failure testosterone luteinizing hormone anaemia uraemic bone disease

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Patients with chronic renal failure commonly have endocrine disorders which contribute to anaemia hypertension premature arteriosclerosis and bone disease Apart from changes in the endocrine activity of the kidneys themselves altered renal function influences the normal function of endocrine glands and the metabolism of their hormones (23) Impairment of spermatogenesis with a normal or slightly increased serum level of follicle stimulating hormone low testosterone and elevated luteinizing hormone (LH) levels in serum has been demonstrated by several authors in males on regular dialysis treatment (RDT) (3 9 13 16 21 25) but little is known about the severity of androgenic metabolic derangement in patients with chronic renal disease not undergoing dialysis treatment Serum levels of testosterone and LH have previously been reported in eight male patients (3 8 12) The present study was undertaken in order to provide further data

Abbreviations LH=luteinizing hormone RDT=regular dialysis treatment GFR=glomerular filtration rate BPR=blood production rate MCR=metabolic clearance rate

PATIENTS

Among some 2800 patients admitted to the Department of Medicine during a period of one year a consecutive series of 33 was selected according to the following criteria 1) Chronic not dialysed renal disease in a relatively stable phase (serum creatinine ≥130 μmol/l) 2) Absence of other serious disease Excluded were subjects with histories of malignant disease chronic respiratory and liver disease endocrine disease disease of possible autoimmune origin and haematological disease apart from the anaemia of chronic renal failure None of the patients had recently undergone major surgery none received steroids or cytostatics nor showed clinical signs of acute infection There were 17 males and 16 females aged 39–84 years (mean 63 6) The clinical diagnoses are given in Table I and the indices of renal failure in Table II

METHODS

Serum concentration of testosterone was measured by a modification of the radioimmunoassay of Furuyama et al (7) after preliminary separation of testosterone by thin

Table I *Clinical diagnoses of the 33 patients studied*

	Males	Females
Chronic glomerulonephritis	2	0
Chronic pyelonephritis	1	5
Chronic interstitial nephritis	1	7
Nephrosclerosis	8	1
Gouty nephropathy	2	0
Nephrocalcinosis	2	1
Non classified	1	2
Total	17	16

Table II *Biochemical indices of kidney disease in 33 patients*

	Males		Females	
	Mean	*Range*	*Mean*	*Range*
Serum creatinine (µmol/l)	328	130–1 312	335	161–1 227
Serum urea (mmol/l)	21 2	5 7–65 8	22 2	8 3–70 1
GFR (ml/min)	36	6–68	19	6–40
Hb (mmol/l)	8 0	5 1–10 2	7 4	5 6–9 9

* Three female patients were excluded because of recent blood transfusions

Table III *Serum levels of testosterone and LH in patients and reference groups (mean±S D)*

	Patients		Controls		p
♂ *Testosterone (nmol/l)*	*16 4*	*6 3*	*22 4*	*5 8*	*<0 01*
LH (log mIU 2nd IRP HMG/ml)	1 08	0 27	0 78	0 21	<0 001
♀ Testosterone (nmol/l)	1 1	0 9	1 8	0 6	<0 01

layer chromatography The method was developed at and the analyses performed by Medi Lab Copenhagen The interassay coefficient of variation was 7% though not less than 0 3 nmol/l Reference intervals of the laboratory ♂ 10 7–34 nmol/l (17 healthy males aged 20–60) ♀ 0 6–3 0 nmol/l (36 healthy females aged 18–55) Serum concentration of LH was measured by a double antibody radioimmunoassay (26) at Statens Seruminstitut Copenhagen The interassay coefficient of variation was 11% Reference interval of the laboratory ♂ 2–16 mIU 2nd IRP HMG/ml (50 healthy males aged 20–50) Glomerular filtration rate (GFR) was estimated as the endogenic creatinine clearance

In the statistical analysis correlation coefficients (*r*) were calculated by the method of least squares Student s *t* test was used to estimate the significance of differences between mean values in patients and reference groups

RESULTS

Table III shows the mean values of testosterone and LH in patients and reference groups Fig 1 the individual values of the patients Mean serum testosterone was decreased in both sexes but only two males and five females had subnormal levels Mean serum LH was increased in the males and eight patients had levels above the reference interval Serum testosterone was inversely correlated with serum urea ($r=-0\ 51$ $P<0\ 01$) (Fig 2) while a positive correlation was found against GFR ($r=0\ 48$ $p<0\ 01$) (Fig 3) Serum LH and GFR were *not significantly* correlated ($r=-0\ 32$ $0\ 1<p<0\ 2$) neither was there a correlation between serum LH and serum urea ($r=0\ 18$ n s) or between serum LH and serum testosterone ($r=0\ 00$ n s) A highly significant correlation was found between Hb and serum testosterone ($r=0\ 60$ $P<0\ 001$) (Fig 4) GFR and the serum levels of testosterone and LH were not related to the age of the patients

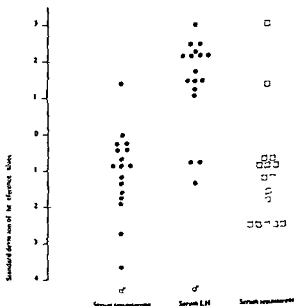


Fig 1 Individual values of serum testosterone and serum LH in 33 patients with chronic renal disease The values are referred to the mean reference values as zero and 1 S D as unit

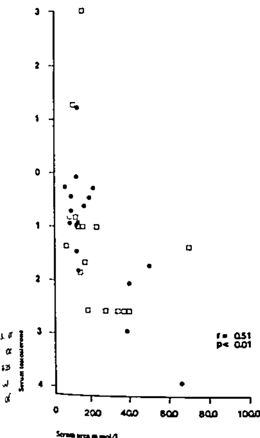


Fig 2 Relation between serum testosterone and serum urea in 33 patients (●=males □=females) Serum testosterone is referred to the mean reference value as zero and 1 S D as unit.

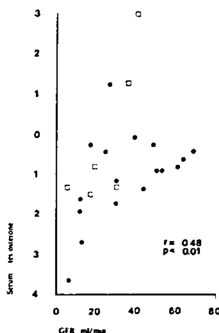


Fig 3 Relation between serum testosterone and GFR in 33 patients (●=males □=females) Serum testosterone is referred to the mean reference value as zero and 1 S D as unit

DISCUSSION

The present results indicate reduction of serum testosterone with decreasing renal function in both sexes (Figs 2 and 3) The difference in age between patients and reference groups could hardly explain this relation Mean serum testosterone level is stable between the ages of 20 and 79 in males (11) and throughout life in healthy females (2)

A reduced blood production rate (BPR) of testosterone with an increase in the metabolic clearance rate (MCR) has been demonstrated in males on RDT (15 30) and both mechanisms were probably responsible for the reduction of serum testosterone in the present patients Reduction of BPR appears to be a consequence of uraemic intoxication and increase in MCR may be explained by a high hepatic plasma flow (4) Changes in the testosterone binding globulin have not been found (3 10)

The increase in serum LH might suggest a hypothalamic pituitary feedback from a low serum testosterone (30) but the present data do not support this assumption since no correlation was found between the serum levels of testosterone and LH LH is eliminated by renal excretion (17) and Holdsworth et al (13) have recently demonstrated a reduced MCR of LH in males on RDT This finding agrees with the weak inverse correlation between serum LH and GFR in the present study The lack of correlation confirms that hypogonadism in uraemic males is a consequence of testicular dysfunction and inadequate hypothalamic pituitary response to the end-organ failure (21)

Androgen production in females takes place in the ovaries the adrenals and through conversion of precursors in non-endocrine tissues (5) A hypoandrogenic syndrome has not been established in females The physiological importance of these steroids is obscure although several actions upon the reproductive organs and protein metabolism are conceivable (27) We have demonstrated some reduction of the serum level of testosterone in azotaemic females not undergoing dialysis treatment and the finding suggests similar changes in

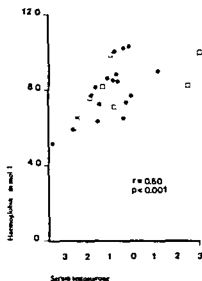


Fig 4 Relation between Hb and serum testosterone in 30 patients (●=males □=females) Serum testosterone is referred to the mean reference value as zero and 1 S D as unit

the quantitatively more important adrenal androgens

Little is known about the clinical significance of the changed serum levels of testosterone and LH Impotence loss of libido and subfertility are common in advanced uraemia (10 19 28) but these do not correlate with the serum levels of ...sterone (10) and neurologic as well as mental ...ances have to be considered (28) Antoniou et al (1) have emphasized zinc deficiency as a reversible cause of gonadal dysfunction in uraemia and hyperparathyroidism may be of some importance (24)

The erythropoietic potency of androgens is surpassed only by erythropoietin (6 27) The results presented here suggest some relation between anaemia of chronic renal failure and deficiency in endogenous androgen (Fig 4) This subject has not been treated in the literature The therapeutic use of androgens in pharmacological doses is controversial (20)

Disturbed vitamin D metabolism is of major importance in azotaemic bone disease (18) Nevertheless *androgen deficiency is a risk factor in post* menopausal osteoporosis (14 22) and a similar influence upon the azotaemic bone disease is conceivable

There is probably no disadvantage in giving physiological doses of androgens to patients with chronic renal disease, whether it is rational must await further investigation

REFERENCES

1 Antoniou L Shalhoub R J Sudharkar T & Smith J C Reversal of uraemic impotence by zinc Lancet 2 895 1977
2 Chakravarti S Collins W P Forecast J D Newton J R Oram D H & Studd J W W Hormonal profiles after the menopause Br Med J 2 784 1976
3 Chen J C Vidt D G Zorn E M Hallberg M C & Wieland R G Pituitary Leydig cell function in uremic males J Clin Endocrinol 31 14 1970
4 Corvol P Bertagna X & Bedrossian J Increased steroid metabolic clearance rate in anephric patients Acta Endocrinol (Kbh) 75 756 1974
5 Drucker W D Biologic activity and metabolism of androgenic hormones The role of adrenal androgens Bull NY Acad Med 53 347 1977
6 Fried W Erythropoietin Arch Intern Med 131 929 1973
7 Furuyama S Mayes D M & Nugent C A A radioimmunoassay for plasma testosterone Steroids 16 415 1970
8 Guevara A Vidt D Hallberg M C Zorn E M Pohlman C & Wieland R G Serum gonadotropin and testosterone levels in uremic males undergoing intermittent dialysis Metabolism 18 1062 1969
9 Gupta D & Bundschu H D Testosterone and its binding in the plasma of male subjects with chronic renal failure Clin Chim Acta 36 479 1972
10 Hagen C Ølgaard K McNeilly A S & Fisher R Prolactin and the pituitary gonadal axis in male uraemic patients on regular dialysis Acta Endocrinol (Kbh) 82 29 1976
11 Haug E Aakvaag A Sand T & Torjesen P A The gonadotrophin response to synthetic gonadotrophin releasing hormone in males in relation to age dose and basal serum levels of testosterone oestradiol 17 β and gonadotrophins Acta Endocrinol (Kbh) 77 625 1974
12 Heinrichs H R Milde K Junkers K Heinze V & Burmeister P Untersuchungen zum Testosteronspiegel im Plasma mannlicher Patienten unter Chronischer Hamodialysebehandlung Verh Dtsch Ges Inn Med 79 734 1973
13 Holdsworth S Atkins R C & de Kretser D M The pituitary testicular axis in men with chronic renal failure N Engl J Med 296 1245 1977
14 Hollo I Szalay F Szucs J & Boross M Osteoporosis and androgens Lancet 1 1357 1976
15 van Kammen E Thyssen J H H & Schwarz F Androgen metabolism in uraemic men (abstract) J Endocrinol 64 49P 1975
16 de Kretser D M Atkins R C Hudson B & Scott D F Disordered spermatogenesis in patients with chronic renal failure undergoing maintenance haemodialysis Aust NZ J Med 4 178 1974
17 de Kretser D M Atkins R C & Paulsen C A

Role of the kidney in the metabolism of luteinizing hormone J Endocrinol 58 425 1973
18 Kroiner B Schaadt O Sanvig Christensen M Clausen E Lund B Melsen F Pors Nielsen S & Sorensen O H 1α hydroxy kolekalciferol Ugeskr Laeger 139 880 1977
19 Larsen N A Sexual problems of patients on RDT and after renal transplantation Proceedings of the European Dialysis and Transplant Association Excerpta Med (Amst) 9 271 1972
20 Leading article Androgens in the anaemia of chronic renal failure Br Med J 2 418 1977
21 Lim V S & Fang V S Gonadal dysfunction in uremic men A study of the hypothalamo-pituitary testicular axis before and after renal transplantation Am J Med 58 655 1975
22 Marshall D H Crilly R G & Nordin B E C Plasma androstenedione and oestrone levels in normal and osteoporotic postmenopausal women Br Med J 2 1177 1977
23 Massry S G (ed) Symposium on kidney and hormones Nephron 15 161 1975
24 Massry S G Goldstein D A Procci W R & Kletzky O A Impotence in patients with uremia. A possible role for parathyroid hormone Nephron 19 305 1977
25 Mies R. v Baeyer H Figge H Finke K & Winkelmann W Investigations on pituitary and Leydig cell function in chronic hemodialysis and after renal transplantation Klin Wochenschr 53 611 1975
26 Roos J & Music S Radioimmunologisk bestemmelse af hypofysegonadotropinerne LH og FSH i humant serum Ugeskr Laeger 137 11 1975
27 Shahidi N T Androgens and erythropoiesis New Engl J Med 289 72 1973
28 Sherman F P Impotence in patients with chronic renal failure on dialysis Its frequency and etiology Fertil Steril 26 221 1975
29 Sommerville I F & Collins W P Indices of androgen production in women Adv Steroid Biochem Pharmacol 2 _67 1970
30 Stewart Bently M Gans D & Horton R Regulation of gonadal function in uremia. Metabolism 23 1065 1974

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Iron Absorption and Iron Status in Patients with Chronic Uremia on Regular Peritoneal Dialysis

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ABSTRACT Gastrointestinal iron absorption was measured by whole body counting in 18 patients on regular peritoneal dialysis Ten patients received regular oral iron treatment prior to the study (iron treated group), 8 patients did not receive iron treatment (non iron treated group) Whole body retention 14 days after oral administration of 10 μCi ^{59}Fe together with a carrier dose of 10 mg Fe^{2+} was used as an estimate of absorption The erythrocyte iron incorporation i e the percentage of administered ^{59}Fe incorporated into the total erythrocyte mass, was measured Geometric mean iron absorption in the non iron treated groups was 7 4±3 3 (S D) % and in the iron treated group 2 8±2 5% ($p<0$ 01) Absorption in the non iron treated group did not differ significantly from the value in a normal control group ($p>0$ 3) Absorption in the iron treated group was distinctly lower than in the controls ($p<0$ 01), due to the high iron supplementation Several patients in the non iron treated group had latent or overt iron deficiency, while patients in the iron treated group had satisfactory iron status The correlation between iron absorption and erythrocyte iron incorporation was highly significant ($r=0$ 95, $p<0$ 001) Peritoneal dialysis patients on the whole have a normally functioning iron absorption However, due to increased iron losses and insufficient dietary iron intake, the maintenance of a satisfactory iron balance implies an adequate oral iron supplementation

Key words chronic renal failure peritoneal dialysis iron absorption bone marrow iron oral iron treatment

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In previous investigations we have evaluated iron absorption and iron status in non dialysed patients with chronic renal failure (18) in patients on regular hemodialysis (19) and in renal transplanted patients (20) In these earlier studies we have drawn the conclusion that the absorption of iron in these subjects is well functioning and capable of adapting to the often increased demands for iron

Regular peritoneal dialysis (RPD) has in the last years been accepted as an alternative treatment to regular hemodialysis in selected patients (7) Like other uremic subjects patients on RPD display a more or less pronounced anemia caused mainly by deficient erythropoiesis due to failing production of renal erythropoietic stimulating factor (8) Extra blood losses either iatrogenic through blood sampling or through increased menstrual and gastrointestinal losses (15) contribute to the anemia and predispose to the development of iron deficiency The absence of systematic investigations on iron absorption and iron status in peritoneal dialysis patients has motivated the present study

PATIENTS

Eighteen patients (5 males 13 females) participated in the study (Table I) All had 24-hour endogenous creatinine clearance of ≤5 0 ml/min and had been on RPD during 1 5 to 11 0 months (mean 4 1 months) Six patients (nos 1 2 4 5 9 and 11) were on center dialysis for 30 hours once weekly the other 12 patients underwent home dialysis for 5 hours daily 6 times weekly (7) Dialysis was performed through an indwelling Tenckhoff peritoneal catheter using a manually operated system (7) and a dialysate containing oxytetracycline 12 5 mg/l (center dialysed group) or 25 mg/l (home dialysed group) as a prophylactic against infection In patient no 7 ampicillin was employed instead The center treated and the home treated group were comparable with regards to the duration of RPD creatinine clearance serum creatinine and serum urea

The patients were taking a protein restricted diet containing an average of 0 8 g protein/kg b wt /day and

Abbreviations RPD=regular peritoneal dialysis EIC=erythrocyte iron incorporation MCV=mean corpuscular volume MCHC=mean corpuscular Hb concentration TIBC=plasma total iron binding capacity

Table I. *Clinical and renal data in 18 patients on regular peritoneal dialysis*

Pat. no.	Sex	Age (y.)	Diagnosis	Serum urea (mmol/l)	Serum creatinine (mmol/l)	Creatinine clearance (ml/min)
1	♂	51	Chronic glomerulonephritis	29	1.07	0.4
2	♂	30	Chronic glomerulonephritis	30	1.09	2.9
3	♂	57	Polycystic kidneys	20	1.10	3.0
4	♀	55	Polycystic kidneys	30	0.94	3.3
5	♀	18	Chronic glomerulonephritis	25	1.27	4.9
6	♀	39	Chronic glomerulonephritis	23	1.05	0.7
7	♀	46	Polycystic kidneys (Unilateral nephrectomy)	26	0.99	5.0
8	♀	34	Nephrosclerosis	35	0.93	1.0
9	♂	29	Chronic interstitial nephritis *(Unilateral nephrectomy)*	27	1.49	0.2
10	♂	52	Polycystic kidneys	16	0.80	4.0
11	♀	57	Polycystic kidneys	37	1.97	0.1
12	♀	43	Chronic interstitial nephritis	18	0.88	1.8
13	♀	53	Chronic interstitial nephritis	33	0.87	0.2
14	♀	45	Chronic interstitial nephritis	25	1.03	2.0
15	♀	57	Polycystic kidneys	31	1.19	3.0
16	♀	57	Polycystic kidneys	22	0.95	1.9
17	♀	42	Nephrosclerosis	26	1.34	3.0
18	♀	45	Chronic glomerulonephritis	23	1.07	2.3
Arithmetic mean		45		26	1.11	2.2
S.D.		11		6	0.28	1.6
Normal range				≤7.5	≤0.13	≥80

multivitamin supplements except folate and B_{12}. Aluminium aminoacetate was administered daily in order to correct the hyperphosphatemia. None of the patients had been subjected to gastrointestinal surgery or had symptoms of malassimilation or infection. All patients had direct Coombs test, normal serum bilirubin and nuria ranging from 0.2–5.0 g/24 hours (mean 1.2 g/24). Routine blood transfusions were avoided, but 2 ients (nos. 15 and 16) had received a single series of transfusions 10 and 3 months, respectively, before the study. Five of the females were menstruating (Table II). Blood sampling was restricted to a minimum and there were no unexpected blood losses during the investigation. Ten patients received peroral iron as ferrous fumarate 400 mg (132 mg elemental Fe^{2+}) three times daily (Table II). This treatment was discontinued at least two weeks before the iron absorption study.

The control group consisted of 27 non iron treated healthy subjects (8 males, 19 females) with a mean age of 32±13 (S.D.) years (range 17–69 years). Details about this material have been published earlier (16).

METHODS

Iron absorption was measured by whole body counting using the method described previously (16). All non vital medicine including aluminium aminoacetate, was withheld at least for days before the study. Blood sampling was avoided in the investigation period. Measurements of the whole body ^{59}Fe activity were performed at four hours (100% value) and 14 days after the oral administration of 10 μCi ^{59}Fe together with 9.9 mg Fe^{2+} (as sulphate) as carrier to the fasting subject. After correction for background radioactivity and radioactive decay, the percentage absorption of iron was calculated. *The erythrocyte iron incorporation* (EIC), i.e. the percentage of administered ^{59}Fe recovered in the total erythrocyte mass at the final counting procedure, was measured as previously reported (16).

Hb, serum creatinine and serum urea values represent an average of all predialytic measurements within 1–2 months before the study. Hematologic parameters, including Hb, mean corpuscular vol*ume (MCV), mean corpuscular Hb concentration* (MCHC), corrected reticulocyte count, serum iron, plasma transferrin and plasma total iron binding capacity (TIBC), were estimated by earlier described procedures (16).

Bone marrow specimens were obtained only in RPD patients by sternal or iliac crest puncture, stained for iron with Prussian blue, whereafter the stainable iron content was graded in 5 classes (0–4+). The Institute of Pathology, Rigshospitalet, was at work upon the preparation and evaluation of the marrow aspirates.

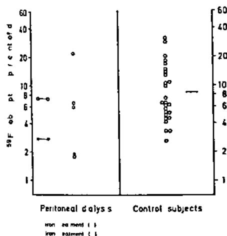


Fig 1 Absorption of ^{59}Fe in patients on regular peritoneal dialysis and in control subjects (geometric mean indicated)

Logarithmic transformation of the values for iron absorption and EIC was employed in the calculation of the geometric mean (5) Regression lines were calculated according to the method of least squares Student s t test for unpaired values and the two-tailed Mann Whitney rank sum test were employed in the statistical evaluation of the results

RESULTS

The iron absorption and EIC values as well as hematological and biochemical data in RPD patients and control subjects are shown in Table II and Fig 1

Iron absorption and erythrocyte iron incorporation

The non iron treated RPD patients had a geometric mean iron absorption of 7 4±3 3 (S D) % and a geometric mean EIC of 5 9±3 2% In the iron treated patients absorption was 2 8±2 5% and EIC 3 5±1 9% (Table II) Iron absorption was significantly higher in the non iron treated than in the iron treated group ($p<0\ 01$)

The control subjects had an iron absorption of 8 5±2 1% (range 1 9–38 3%) and an EIC of 7 7±2 2% (range 1 6–37 0%) Absorption and EIC in the non iron treated group were not significantly different from the control values ($p>0\ 3$ and $p>0\ 08$) whereas absorption and EIC in the iron treated group were definitely lower than in the controls ($p<0\ 01$ and $p<0\ 01$)

Iron absorption in the center dialysed group (4 6±3 0%) was not significantly different from absorption in the home dialysed group (4 2±3 3%)

There was a highly significant correlation between iron absorption and EIC both in patients ($r=0\ 95$ $p<0\ 001$) and in controls ($r=0\ 96$ $p<0\ 001$) as shown in Fig 2

The iron absorption demonstrated a positive correlation with the plasma TIBC ($r=0\ 65$ p 0 01) there was no correlation with serum iron transferrin saturation degree of uremia or duration of dialytic therapy

Hematological studies

Generally the patients on RPD had a normochromic anemia compared to the controls ($p<0\ 05$) Two iron treated patients (nos 10 and 14) had Hb within the normal range The Hb was slightly higher in the iron treated than in the non iron treated group (Table II)

The iron treated group had normal MCV values ($p>0\ 5$) whereas the non iron treated group had MCV values which were significantly lower than in both control subjects ($p<0\ 01$) and iron treated patients ($p<0\ 05$)

The corrected reticulocyte count was normal in the iron treated group ($p>0\ 7$) while the non iron treated group had lower values than both controls ($p<0\ 05$) and iron treated patients ($p<0\ 05$)

On the whole bone marrow examination showed

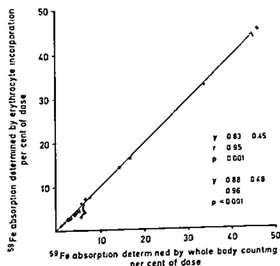


Fig 2 Relation between absorption of ^{59}Fe and erythrocyte incorporation of administered ^{59}Fe in patients on regular peritoneal dialysis (●) and control subjects (O)

Table II *Hematological data iron absorption and erythrocyte iron incorporation in 18 patients on regul peritoneal dialysis and 27 healthy control subjects Patients are divided into two groups according preceding iron treatment*

Pat no	Hb (mmol/l)	Hematocrit	Corrected reticulocyte count (l/1 000)	MCHC (mmol/l)	MCV (fl)	Serum iron (μmol/l)	Plasma transferrin (μmol/l)	Plasma TIBC (μmol/l)	Transferrin saturation %	Marrow iron (0–4+)
1	6 2	0 30	5	19 7	89	17 1	25 2	50 4	33 9	0–1+
2	5 9	0 28	3	20 6	86	15 5	28 2	56 4	27 5	2+
3	5 5	0 24	2	21 7	84	9 5	16 3	32 6	29 1	2+
4	6 6	0 33	12	19 6	80	7 8	16 5	33 0	23 6	1+
5	5 3	0 20	4	20 1	84	10 3	23 7	47 4	21 7	0
6	5 4	0 24	4	22 4	76	10 2	26 0	52 0	19 6	0–1+
7	6 4	0 32	5	20 2	81	8 4	37 5	75 0	11 2	0
8	4 1	0 20	7	21 5	79	5 7	23 8	47 6	12 0	0
m	5 7	0 26	5	20 7	82	10 6	24 7	49 3	22 3	
S D	0 8	0 05	3	1 0	4	3 9	6 7	13 5	8 0	
m_g										
S D										
9	4 8	0 24	4	19 5	83	8 2	25 3	50 6	16 2	1+
10	7 4	0 35	6	22 4	90	11 2	18 7	37 4	29 9	2+
11	5 6	0 24	15	21 0	89	17 7	23 3	46 6	38 0	1+
12	6 8	0 33	5	20 7	87	12 5	25 4	50 8	24 6	0–1+
13	5 7	0 27	–	21 5	91	12 2	26 3	52 6	23 2	0–1+
14	7 2	0 31	21	22 6	93	8 0	22 6	45 2	17 7	3+
15	5 4	0 24	7	22 3	82	11 5	22 6	45 2	25 4	2+
16	6 7	0 33	19	19 0	81	10 0	19 9	39 8	25 1	2+
17	6 0	0 28	10	21 9	83	27 6	26 6	53 2	51 9	3+
18	6 2	0 30	10	21 2	93	12 6	22 9	45 8	27 5	2+
m_g	6 2	0 29	11	21 2	87	13 2	23 4	46 7	28 0	
S D	0 8	0 04	6	1 2	5	5 8	2 6	5 3	10 4	
	1 subjects (16) (n = 27)									
m	8 5	0 41	10	20 9	88	20 3	32 6	65 1	31 8	
S D	0 7	0 04	6	0 9	4	7 0	6 4	12 8	10 7	
m_g										
S D										

m = arithmetic mean m_g = geometric mean

normoplastic marrow and in all patients the erythropoiesis was normoblastic In the non iron treated group the stainable marrow iron content was normal in 3 patients 2 patients had only traces of iron and the staining was negative in 3 patients In the iron treated group marrow iron was normal or abundant in 8 patients while only traces were present in 2 patients The marrow iron score was significantly higher in the iron treated than in the non iron treated group ($p<0$ 01)

Biochemical studies

Both the non iron treated and the iron treated RPD group had significantly lower serum iron ($p<0$ 01 and $p<0$ 01) and plasma transferrin ($p<0$ 01 and $p<0$ 01) than the control group The transferrin saturation was significantly lower in the non iron treated group compared to the controls ($p<0$ 05) while the iron treated group had a normal saturation ($p>0$ 2) The non iron treated group had slightly lower serum iron and transferrin saturation and higher plasma TIBC than the iron treated group (Table II)

Serum iron plasma TIBC and transferrin saturation displayed considerable variations and were poorly correlated to stainable marrow iron stores Thus 5 patients (nos 3 4 9 14 and 16) with normal iron stores had subnormal serum iron while two

Fe absorption (%)	^{59}Fe erythrocyte incorporation (%)	Preceding iron treatment (mo)	Menstruation (+)
1 4	19 6	0	
1 8	1 8	0	
6 5	3 2	0	
1 3	1 3	0	
5 7	6 2	0	+
[illegible] 8	14 4	0	+
33 4	32 9	0	+
6 0	3 6	0	+
[illegible] 5	10 4	0	
12 0	11 2		
7 4	5 9		
3 3	3 2		
12.5	6 5	4	
3 8	6 6	3	
2 7	2 4	3	+
5 9	5 2	3	
7 6	7 5	6	
3 2	2 5	12	
1 9	3 5	12	
1 0	2 5	4	
1 0	1 0	36	
0 9	3.5	3	+
4 1	4 1		
3 7	2 2		
2 8	3 5		
2 5	1 9		
11 5	10 6	0	
10 4	9 6		
8 5	7 7		
2 1	2 2		

patients (nos 12 and 13) with only traces of marrow iron had normal serum iron

DISCUSSION

Several factors predispose to a negative iron balance in RPD patients such as 1 Blood sampling for routine controls and research the estimated average minimum blood loss in the RPD group was 20 ml/mongh 2 Increased menstrual and gastrointestinal blood losses (15) 3 Increased urinary iron loss (6) 4 Probable loss of transferrinbound iron during dialysis 5 Low iron content in the protein restricted diet (18) 6 Unfavorable iron absorption conditions from a diet poor in meat and blood products (4 17) 7 Administration of aluminium aminoacetate which probably compromises iron absorption, although the reports hereon are somewhat contradictory (3 21)

Iron absorption in the non iron treated RPD group was of the same order of magnitude not significantly differing from absorption in the control group Although absorption in the non iron treated group was slightly lower than could be expected according to the above considerations the presented results suggest that iron absorption on the whole is normally functioning in RPD patients and exclude significant absorption defects The apparently low absorption results might partly be explained by the low sensitivity of the iron absorption test due to the relatively high carrier dose employed (10)

The distinctly lower absorption in the iron treated group is undoubtedly a result of the intense iron treatment inasmuch as iron status was satisfactory and none of the patients showed unequivocal signs of iron deficiency

Iron absorption in the non iron treated group was *not significantly different from the earlier obtained* results with a similar technique (8 5±1 7%) in non iron treated non dialysed chronic uremics with iatrogenic blood losses of the same order of magnitude (18)

Like in previous investigations (16 18 19 20) there was a highly significant correlation between the iron absorption and the EIC (Fig 2) The measurement of EIC was primarily intended as a sort of check on iron absorption values The close connection between these two variables emphasizes the reliability of the whole body counting method in the assessment of iron absorption

Three of the five patients in the non iron treated group with depleted marrow iron stores had absorption values above 21% while 2 patients (nos 5 and 8) *had unexpectedly low values* Besides negative marrow iron staining patient no 5 had no other indications of iron deficiency whereas patient no 8 was clearly iron deficient (lowest Hb of all subjects microcytosis very low serum iron and transferrin saturation) In the latter patient hypo- or achlorhydria might possibly account for the low absorption (4) However we did not measure gastric acid output and there exists no study which in general clarifies this subjects in RPD pati

We have also considered th

of tetracycline on iron absorption Tetracycline is to some extent absorbed systemically from the dialysate (22) and is known to inhibit iron absorption through the formation of insoluble chelates (11) Serum oxytetracycline was measured by microbiological assay in some of the patients The center dialysed group had predialytic levels around 0 3 μg/ml and postdialytic levels around 4 μg/ml while the home dialysed group had interdialytic levels around 1 μg/ml Oxytetracycline is in uremic subjects excreted mainly through the bile (24) and could hereby compromise the iron absorption However calculations on the bile concentration of oxytetracycline (24) compared to the iron test dose render it probable that the influence on absorption is negligible

The plasma transferrin values were subnormal in the RPD patients and lower than in the previously investigated non dialysed and hemodialysed uremic subjects (18 19) These very low transferrin levels are probably caused by a restricted protein intake in combination with transferrin losses during dialysis (2) and through the gastrointestinal tract (14) Furthermore the RPD group had significantly lower albumin levels than both the non-dialysed and hemodialysed uremics but there was no correlation between plasma transferrin and plasma albumin in any of the three groups

The poor correlations between stainable marrow on and serum iron plasma TIBC and transferrin saturation are consistent with observations from our previous studies (18 19 20) In part this could be explained by the inaccuracy of the semiquantitative histochemical method employed in the assessment of marrow iron stores We had no opportunity to perform chemical analysis of the non heme marrow iron concentration which provides a better measure of iron stores (23) Furthermore one must recognize that such estimations of iron stores yield no information concerning the availability of the demonstrated iron for erythropoiesis inasmuch as impaired reticuloendothelial iron release has been demonstrated in uremic subjects (1) Serum ferritin is closely correlated to iron stores in normal subjects (13) and hemodialysis patients (12) Sequential monitoring of this protein will probably give a more reliable picture of iron balance (9)

The iron treated group had a satisfactory and definitely better iron status and consequently a distinctly lower absorption than the non iron treated group in which several patients displayed either latent or overt iron deficiency Obviously, in most RPD patients dietary iron intake alone is insufficient in sustaining a satisfactory iron balance especially in menstruating females (Table II)

Besides showing that iron absorption generally is normally functioning in peritoneal dialysis patients this study demonstrates the need for as well as the efficiency of oral iron supplementation in maintaining a satisfactory iron status thus preventing deficiency symptoms We suggest a dose of ferrous fumarate 200 mg 3–4 times daily to males and non menstruating females and a double dose to menstruating females Parenteral iron administration should be reserved for patients with malassimilation or patients in whom oral iron treatment for some other reasons has failed Iron status including assessment of marrow iron stores should be monitored at reasonable intervals

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REFERENCES

1 Beamish M R Davies A G Eakins J D Jacobs A & Trevett D The measurement of reticuloendothelial iron release using iron-dextran Br J Haematol 21 617 1971
2 Berlyne G M Hewitt V Jones J H & Nilwarangkur S Protein loss in peritoneal dialysis. Lancet 1 738 1964
3 Blumberg A Der Einfluss aluminiumhydroxydhaltiger Antazida auf die enterale Eisenresorption von Langzeitdialysepatienten Schweiz Med Wochenschr 107 1064 1977
4 Conrad M E Factors affecting iron absorption In Iron deficiency (ed L Hallberg H G Harwerth & A Vanotti) pp 87–120 Academic Press London and New York 1970
5 Cook J D Layrisse M & Finch C A The measurement of iron absorption Blood 33 421 1969
6 Dagg J H Smith J A & Goldberg A Urinary excretion of iron Clin Sci Mol Med 30 495 1966.
7 Dawids S G & Christensen E Chronic home peritoneal dialysis with a simple dialysis system Proc Eur Dial Transplant Assoc 12 149 1976
8 Erslev A J Anemia of chronic renal disease Arch Intern Med 126 774 1970
9 Eschbach J W Cook J D Schribner B H & Finch C A Iron balance in hemodialysis patients. Ann Intern Med 87 710 1977
10 Heinrich H C Intestinal iron absorption in man. In Iron deficiency (ed L Hallberg H -G Harwerth & A Vanotti) pp 213–296 Academic Press London and New York 1970

11 Heinrich H C Oppitz K H & Gabbe E E Hemmung der Eisenresorption beim Menschen durch Tetracyclin Klin Wochenschr 52 493 1974

12 Hussein S Prieto J O Shea M Hoffbrand A V Baillod R A & Moorhead J F Serum ferritin assay and iron status in chronic renal failure and hemodialysis Br Med J 1 546 1975

13 Jacobs A Serum ferritin and iron stores Fed Proc 36 2024 1977

14 Johansson S V Odar Cederlof I Plantin L O & Strandberg P O Albumin metabolism and gas trointestinal loss of protein in chronic renal failure Acta Med Scand 201 353 1977

15 Koch K M Bechstein P B Fassbinder W Kaltwasser P Schoeppe W & Werner E Occult blood loss and iron balance in chronic renal failure Proc Eur Dial Transplant Assoc 12 362 1976

16 Larsen L & Milman N Normal iron absorption determined by means of whole body counting and red cell incorporation of ^{59}Fe Acta Med Scand 198 271 1975

17 Martinez Torres C & Layrisse M Iron absorption from veal muscle Am J Clin Nutr 24 531 1971

18 Milman N & Larsen L Iron absorption in patients with chronic renal failure not requiring dialytic therapy Acta Med Scand 198 511 1975

19 Milman N & Larsen L Iron absorption in patients with chronic uremia undergoing regular hemodialysis Acta Med Scand 199 113 1976

20 Milman N & Larsen L Iron absorption after renal transplantation Acta Med Scand 200 25 1976

21 Rastogi S P Padilla F & Boyd C M Effect of aluminium hydroxide on iron absorption J Arkansas Med Soc 73 133 1976

22 Ruedy J R The effects of peritoneal dialysis on the physiological disposition of oxacillin ampicillin and tetracycline in patients with renal disease Can Med Assoc I 94 257 1966

23 Weinfeld A Iron stores In Iron deficiency (ed L Hallberg H G Harwerth & A Vanotti) pp 329–363 Academic Press London and New York 1970

24 Weinstein L Antimicrobial agents Tetracyclines and chloramphenicol In The pharmacological basis of therapeutics (ed L S Goodman & A Gilman) 5th edition pp 1183–1200 MacMillan Publishing Co New York 1975

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Tuberculosis and Kidney Transplantation

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ABSTRACT Immunosuppressive treatment enhances the risk of pulmonary and other infections Tuberculosis is a predictable complication in transplanted patients The present material comprises 584 kidney transplantation patients, ten of whom had had a previous history of tuberculosis After transplantation ten patients presented with pulmonary tuberculosis during immunosuppressive treatment One of the patients to whom no prophylactic antituberculous treatment had been given presented with a relapsing tuberculosis The results of routine antituberculous treatment were excellent, but graft and patient survival were disappointing among the tuberculosis patients

Key words immunosuppression kidney transplantation tuberculosis

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Dialysis treatment of patients suffering from severe renal failure is common practice Kidney transplantation has become an accepted and widely spread method of treating terminal renal disease These patients often have severe uremic complications during dialysis They need continuous medication for the treatment of the underlying disease and for the uremic symptoms as well Pradhan et al (9) report five cases of acute tuberculosis among 136 patients on chronic dialysis in New York between 1963 and 1973 This represents an incidence of 15 times that in any normal population

Transplantation requires added immunosuppressive treatment of the already compromised patient (11) which enhances the risk of complicating infections Neff and Hudgel (8) have seen tuberculosis in three renal transplant patients out of 400 between 1962 and 1972 Stake and Flatmark (10) report 18 cases of pulmonary infections in a Norwegian material of 77 transplantations Eleven of these patients died mostly due to opportunistic bacteria Pneumocystis carinii cytomegalovirus and fungi One case of tuberculosis was later reported

PATIENTS AND METHODS

Our research represents the total Finnish kidney transplantation material It comprises 678 transplantations in 584 patients from 1964 to the end of May 1978 In 80% of the cases there was an initial period of dialysis for a mean of 9 months All the patients were treated with immunosuppressive agents during and after transplantation The chemical agents used were azathioprine (Imurel®) 50–150 mg daily and methylprednisolone (Medrol®) initially 60 mg daily in declining doses to 8–36 mg every second day respectively Methylprednisolone 1 g was given every second day to a total of 3–4 g during periods of rejection

A chest X ray was taken at the beginning of dialysis treatment and/or at transplantation Later check ups were performed with intervals of 3–12 months In case symptoms of infection or pulmonary involvement presented sputum specimens were obtained for culture and smears and the lungs were X rayed No tuberculin tests were performed

If tuberculosis was confirmed normal antituberculous therapy was initiated i e 450–600 mg rifampicin (RMP) daily 300 mg isoniazid (INH) daily and 750–1 000 mg streptomycin (SM) daily or 15 mg/kg of body weight ethambutol (EMB) daily SM was not given to patients with renal insufficiency Serum concentrations of INH (7) and EMB (2) were studied to check the possible cumulative effect of these drugs when renal insufficiency was present

Ten patients out of the total of 584 had a history of tuberculosis which motivated particular alertness and preferably the administration of prophylactic INH treatment

RESULTS

After transplantation ten cases of pulmonary tuberculosis were found four women aged 42–64

Abbreviations EMB = ethambutol INH = isoniazid RMP = rifampicin SM = streptomycin RA = rheumatoid arthritis

Table I *Dialysis period transplantation and onset of tuberculosis*

Case no	Dialysis period (mo)	Transplantation date (mo /y)	Onset of TB (mo after transplantation)
1	7	I/73	14
2	6	VI/73	23
3	–	IX/73	16
4	–	IV/74	6
5	12	VI/74	1
6	5	III/75	6
7	12	V/75	32
8		IX/75	
	29	V/76	30
		III/78	
9	13	XI/76	11
10	2	X/77	2

a months after first transplantation

Table III *HLA types and match grade*

Pat no	HLA types	Match
1	A2 3 B7 w35	B
2	A11 w19(=32) B27 w35 Cw1	A
3	A2 3 B27 w35	C
4	A2 B15 40	C
5	A3 B27 w35 Cw1	C
6	A2 3 B7 13	B
7	A1 2 B8 27 Cw1	C
8*	A2 11 B40 w35 w6 C	D
		D
		B
9	A2 11 B12 15 w4 w6 Cw3	D
10	A2(w19) B15 18 w6 Cw3 w4	B

* Three transplantations

years six men aged 30–67 years Six patients had bacteriologically verified disease (one disseminated) three were TB negative but had positive chest X ray changes In one patient who died of unrelated disease before tuberculosis was verified autopsy revealed the disease Analysis of the ten patients shows that only one had a previous history of tuberculosis His pulmonary infiltrates pro- after transpl intation He had not received ylactic INH treatment

Underlying disease

The nephrological diagnosis was based on clinical and radiological evidence and on previous kidney biopsies and/or nephrectomy specimens Three patients suffered from chronic pyelonephritis two had chronic interstitial nephritis one had glomerulonephritis one RA with amyloidosis one polycystic kidneys one primary hyperparathyreoidism with nephrocalcinosis and one had diabetic nephropathy

Table II *Ten patients at the end of the study*

Pat no	Antituberculous treatment	State of patient
1	8 mo according to plan	Died IV/77
2	12 mo according to plan	Died XI/76
3	12 mo according to plan	Well
4	12 mo according to plan	Transpl ectomy X/77
5	12 mo according to plan	Chronic rejection
6	No antituberculous treatment initiated	Died IX/75
7	3 mo ad mortem	Died IV/78
8	3 mo Continuing	Transpl ectomy V/78
9	7 mo Continuing	Well
10	5 mo Continuing	Well

Dialysis

Dialysis treatment had been given for 2–13 months in seven cases before transplantation In one patient the dialysis period was 29 months with short breaks due to three transplantations Two patients were transplanted without initial dialysis (Table I)

Onset of tuberculosis

Tuberculosis was verified 1–32 months after transplantation (Table I) The disease presented with fever in eight cases cough or other respiratory symptoms in three pleural effusion in two All ten had chest X ray changes compatible with tuberculosis

Treatment

The duration of treatment was planned for 8–12 months Three patients had been treated for three five and seven months respectively at the end of the study in May 1978 In one patient therapy was never started No specific side-effects were noted Serum concentrations of INH and EMB did not show any dangerous cumulation even in five patients with marked renal failure Three of these received dialysis treatment regularly The regression of pulmonary infiltrates was satisfactory in all patients All TB positive cases converted to nega

tive Four of the ten tuberculosis patients had died before the end of May 1978 Autopsy did not reveal active tuberculosis in any of the treated patients One of them had died before treatment had been initiated one during treatment and two patients 3 and 4 months respectively after the termination of adequate antituberculous treatment Two patients had had their grafts removed and were receiving dialysis treatment One had a chronic rejection Three were well and had a normal graft function (Table II)

HLA type and match grade HLA type and match grade are seen in Table III

DISCUSSION

Our transplantation material showed a one year patient survival of 64% for necrokidneys and a graft survival of 48% which correlates with international results (4)

Infection is the most common transplantation complication In our material infections were recorded in 41% in accordance with a similar material from Gothenburg where the figure was 40% (1) In our material infection is the main cause of death in 25–30% of transplanted patients About half of these deaths are due to pneumonias caused by opportunistic bacteria and fungi It was predictable that tuberculosis would be found in our transplantation material

Tuberculosis morbidity in Finland was 145/100 000 in 1964 and 79/100 000 in 1975 Our series of 10 out of 584 shows a considerable overrepresentation of tuberculosis Activation of known infiltrates was noted in one case only which may be a result of the INH prophylactic treatment given to the other patients with a history of tuberculosis The International Union against Tuberculosis has shown the favourable effect of INH prophylaxis in other conditions (5) In Finland INH prophylactic treatment is not given routinely to tuberculin positive persons About 90% of the Finnish population have a positive tuberculin skin reaction (3) due to our vaccination policies and possibly to our high rate of infection

The risk of infection is increased during immunosuppressive and rejection treatment There is a potential risk of infecting other transplanted patients and dialysis patients in the ward Tuberculosis was not epidemic in this material however the cases appear regularly with a pause in 1976 (Table I) We have had difficulties with opportunistic bacteria and there was an outbreak of fungus disease in 1975 after which the corticosteroid dosage was reduced (6) HLA typing of the patients showed an overrepresentation of genes A2 A11 and B27 (Table III) compared with the Finnish population in general We do not yet know whether this is important for the patients contracting tuberculosis or nephrological disease or both

Renal patients are regularly checked for infections during dialysis treatment Chest X ray controls of dialysis patients or transplanted patients should not be performed too often We hold the opinion that three months is a sufficient minimum and one year a suitable maximum interval between X rays in uncomplicated cases without respiratory symptoms We did not find any tuberculosis during dialysis treatment but one patient not included in this series presented with glandular tuberculosis a few days before her transplantation

Tuberculosis treatment regimens of kidney transplanted patients did not differ from those of regular tuberculosis patients The results of chemotherapy were good Follow up of serum drug concentrations rendered medication more safe

Tuberculosis was a noteworthy complication in transplanted patients receiving immunosuppressive treatment Complications were common and graft survival was disappointing in our ten tuberculosis patients The disease was however diagnosed through regular check ups and successfully treated with prompt and adequate antituberculous treatment

REFERENCES

1 Brynger H Bitter Suermann H Gabel H Ahlmen J Blohme I Gustafsson Å & Gelin L E Complications after renal transplantation Scand J Urol Nephrol (Suppl) 38 113 1977
2 Froseth B *Preliminary report on a modified method* for determination of ethambutol in serum Scand J Respir Dis (Suppl) 69 81 1969
3 Haro A S Long term evaluation of mass-BCG vaccination campaign A study of thirty years of experience in Finland Tuberculosis and Respiratory Diseases Yearbook vol 6 1977
4 Jacobs C Brunner F P Chantler C Donckerwolcke R A Gurland H J Hathway R A Selwood N H & Wing A J Combined report on regular dialysis and transplantation in Europe VII 1976 Proceedings of the European Dialysis and Transplant Association 14 3 1977

5 Krebs A The IUAT trial on isoniazid preventive treatment in persons with fibrotic lung lesions Proceedings of the XXIIIrd International Tuberculosis Conference 51 no 1 193 1976
6 Kuhlback B & Lalius P Delayed renal and extra renal complications following primarily successful renal transplantation Scand J Urol Nephrol (Suppl) 42 170 1977
7 Maher J R Whitney J M Chambers J S & Stanonis D J The quantitative determination of isoniazid and para aminosalicylic acid in body fluids Am Rev Tuberc 76 852 1957
8 Neff T A & Hudgel D W Miliary tuberculosis in a renal transplant recipient Am Rev Respir Dis 108 677 1973
9 Pradhan R P Katz L A Nidus B D Matalon R & Eisinger R P Tuberculosis in dialyzed patients JAMA 229 798 1974
10 Stake G & Flatmark A Lung complications during immunosuppressive treatment in renal transplant recipients Scand J Respir Dis 57 51 1976
11 Williams D M Krick J A & Remington J S Pulmonary infection in the compromised host Part II Am Rev Respir Dis 114 3 593 1976

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Serum Ferritin During Infection

A Longitudinal Study in Renal Transplant Patients

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ABSTRACT In order to follow the dynamics in the reaction of iron kinetic variables to acute infection 8 renal transplantation patients were followed with test samples every second or third day for about two months It was found that they just as previously shown in otherwise healthy subjects, responded to acute infection with a rise in serum ferritin levels sometimes to very high values In most cases the ferritin elevation started within two days after the onset of fever The peak was reached within a week except when very high values were obtained The fall in serum ferritin after recovery from infection was much faster than in previously investigated groups of patients the plasma half disappearance time for ferritin in one case was but 1 5 days Transferrin did not change in response to infection The expected fall in serum iron during infection was often absent and sometimes obscured by unexpected, sharp peaks in serum iron which bore a temporal relationship to episodes of transplant rejection in 7 of 12 cases

Key words ferritin in serum infection renal transplantation

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A rise in serum ferritin levels during inflammation has been reported by several authors (1 5 10) and its duration studied in patients hospitalized for acute infection was found to last several weeks (2) However the initial phase of the ferritin rise has not been thoroughly investigated

For the present study we selected a group of patients hospitalized for renal transplantation The patients were expected to get infectious complications and could be followed in a longitudinal study without any inconvenience to the patient since blood sampling was performed very often in their routine care The ferritin iron transferrin and haptoglobin levels in serum were measured during the postoperative period to study any relationship between changes in these levels and the course of an acute infection

PATIENTS

Eight patients who underwent renal transplantation with a cadaver kidney were followed from the day of operation and during the hospital stay mostly for about 60 days The transplantation and the following tests were carried out under the care of Dr Karl Erik Fjellstrom and his staff at the Renal Transplant Unit

Serum samples were collected from the routine blood samples taken at 8 a m every second or third day All patients received immunosuppressive therapy prednisolone mean daily dose 0 7 mg/kg b w and azathioprine mean daily dose 2 0 mg/kg b w Additional rejection therapy was given for 12 episodes of suspected transplant rejection It consisted of irradiation 150 rad×3 against the transplant plus SoluMedrone® i v in the doses given in the figures

All patients had a slowly progressive renal disease with gradually decreasing glomerular filtration rate during the years prior to operation Four patients had chronic glomerulonephritis two diabetic nephropathia one nephrosclerosis and one chronic pyelonephritis None had anaemia requiring blood transfusions Only three of the patients had been on chronic haemodialysis before operation There were no surgical postoperative complications

All patients had at least one episode of infection during the study There were 18 episodes of acute infections and a positive isolation of bacteria or virus could be made in eight cases Three of the others showed pulmonary infiltration of an uncertain nature three were typical herpes simplex infections and in the last three no clue to a diagnosis could be found in spite of fever and malaise (Table I)

METHODS

Serum ferritin was measured by the radioimmunoassay method of Wide and Birgegard (13) using ferritin labelled with ¹²⁵I by conjugation with an iodinated propionic acid ester according to the principles of Bolton and Hun Results were expressed in arbitrary units/l. value for a group of 25 normal men was set

Table I Diagnosis in the 18 episodes of acute infection after renal transplantation

Diagnosis	No of pats	Case no	Special investigations
Infection of operation wound	4	8 2 4 5	Pos culture
Herpes simplex	4	8 1 2 4	Pos virus isolation in one patient
Pulmonary infiltration	3	7 1 2	Pos X ray
Septicaemia	2	6 4	Pos blood culture
Urinary tract infection	1	3	Pos urinary culture
Upper respiratory tract infection	1	1	
No diagnosis	3	3 7×2	

(range expressed as 95% limits 40–246) The reasons for using arbitrary units were several there is no international reference standard preparation for ferritin Consequently the normal range varies considerably between laboratories We had no evidence for the homogeneity of the ferritin preparation used Incidentally our normal range corresponds well with that given in μg/l by several investigators (4 6 7 12)

The inter plus intra assay variation calculated as the coefficient of variation from nine repeated assays of control samples on three different levels of ferritin concentration was 8% for 19 arb U/l 6 5% for 72 arb U/l and 4 4% for 160 arb U/l The three control samples are included in every assay

Serum iron estimation was carried out with the routine of the Department of Clinical Chemistry at the hospital according to the principles of Richterich (9) By use of a detergent (Teepol 610) iron was released from transferrin then reduced with sodium ditionite ($Na_2S_2O_4$) after which the E_{546} of a complex with batofenatrolin-disulphonic acid was measured The coefficient of variation was 4% within the normal range

Transferrin and haptoglobin were measured by a nephelometric method according to the principles of Lizana and Hellsing (8) The coefficient of variation within the normal range was 2 and 3% respectively

RESULTS

Results are presented in figures 1 and 2 showing ferritin iron and transferrin in serum as well as body temperature in the 8 patients Serum ferritin fluctuated markedly during the study (Figs 1 and 2) showing a number of peaks as well as long standing elevations After the operation ferritin fell slowly during 2–3 weeks to a base level in 4 of the patients In the other 4 no such fall was observed all the last mentioned had infections during that period An analysis of the time relationship between rises in serum ferritin and the occurrence of infection showed that serum ferritin rose within a few days ($M=2$ 2) after the first elevation in temperature Only in case 7 there was a clear rise in temperature during the study without a ferritin reaction within the next three days On the other hand in case 8 serum ferritin rose a couple of days after the onset of a herpes simplex infection without fever

Thus the serum ferritin levels were elevated in two different situations 1) postoperatively with or without infection 2) during infection at any time during the study If the patient did not get an infection the ferritin level fell from the postoperative level either immediately or after few days and remained low until an infection occurred The postoperative elevations (in the absence of infection) did not exceed 400 arb U/l

Serum ferritin levels were elevated during infections of various kinds bacterial as well as viral Some extremely high values were found 4–50[illegible] arb U/l If the infection was treated with success and the patient was afebrile the ferritin level fell In many cases though, there were repeated or persistent infections and those patients also had persisting ferritin elevations (Figs 1 and 2)

The time from the peak ferritin to half this value varied between 1 5 and 14 days ($M=6$ 8±5 0(S D) The infection induced serum ferritin elevation was not apparently influenced by concomitant high dose cortisone therapy Cases 4 2 1 7 and 6 had ferritin elevations during or shortly after transplant rejection therapy in cases 4 and 1 to very high levels Transplant rejection did not cause a serum ferritin elevation by itself since 8 episodes of rejection were not associated with a rise in ferritin The serum iron curves showed a number of peaks and the expected normal fall in serum iron in the early phase of an infection was in many cases obscured or absent

A time relation was found between some of the serum iron peaks and transplant rejection There were 12 episodes of transplant rejection in the patients One to 5 days ($M=3$ 5) after the start

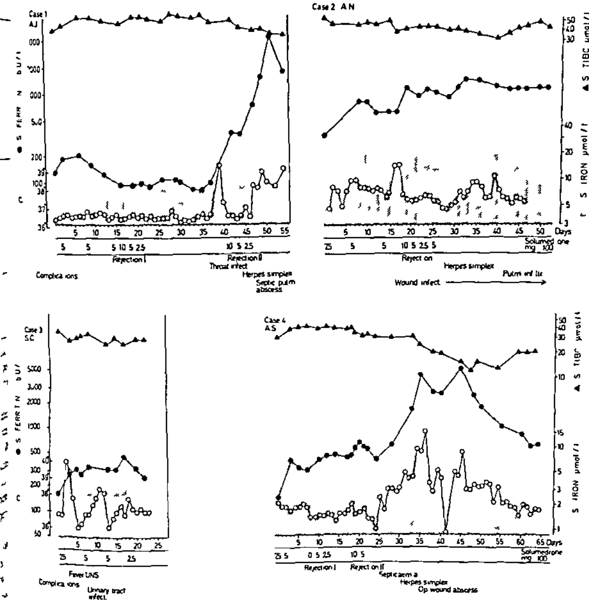
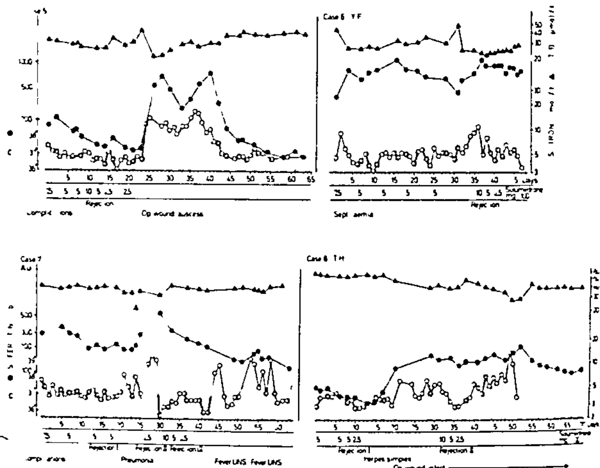


Fig 1 S-ferritin S-TIBC S iron and body temperature in cases 1–4 The first letter in the diagnosis of complications is placed on the time scale on the first day of the episode Time for and doses of rejection cortisone therapy are given Serum iron is shown in bars for the sake of clarity to avoid intersecting curves Each bar represents one observation

such an episode there was in 7 cases a rise in serum iron reaching a peak after 3–5 days ($M=5\ 4$) and of very varying magnitude (4–41 $\mu M/l$ increase $M=17$ $\mu M/l$) In the 5 cases of rejection without an ensuing serum iron peak a lower total dose of hydrocortisone had been given 750 1000 1550 and 1500 mg respectively compared to the usual 1750–2250 mg No other systematic correlations or time relations could be found to the serum iron peaks

Serum haptoglobin was measured but in many cases the pattern seen during infection was not the usual acute phase reaction Several very rapid falls in haptoglobin to subnormal levels during infectio occurred in most cases with a quick return ruled out a comparison with the ferritin cu



2 The same information as in Fig 1 for the following 4 patients

DISCUSSION

In a previous paper (2) we have shown that the serum ferritin elevation during acute infection is of long duration in some cases more than 5–6 weeks even if the patient recovers quickly from the infection It was suggested that the rise in serum ferritin was caused by an augmentation of ferritin synthesis rather than by a release of ferritin from inflammatory cells Since the former study did not include the initial phase of the course of illness on this occation we selected a group of already hospitalized patients with frequent blood samples as part of routine care and liable to get a variety of infectious complications renal transplant patients

It was shown that these patients also had elevated serum ferritin levels during infection of various kinds bacterial as well as viral The serum ferritin started to rise within one or two days after the temperature rise in most cases It must be borne in mind however that the patients were on immunosuppressive therapy which is known to suppress fever reactions The peak ferritin value was reached within a week from the onset of fever except in two cases with extremely high values where the peak was reached after 10 and 14 days As to the ferritin reaction there was a major difference between the present patients and the previously studied the fall in serum ferritin after the peak was much faster here In the extreme case (no 5) only 1 5 days had elapsed before the peak value of 750 arb U/l had fallen to 375 arb U/l The reason for this is uncertain It does not seem likely that the large ferritin molecule could be cleared with the urine especially since there was no correlation to the degree of impairment of the renal transplant Thus the rapid fall in serum ferritin in some cases indicates that the plasma half disappearance time of ferritin was shorter than 2–3 days If a stimulation of ferritin synthesis was responsible for the rise in

serum ferritin during infection at least in these patients it was not maintained maybe because of the immunosuppressive therapy which is known to suppress protein synthesis It is also possible that *the rise in serum ferritin during infection in these* patients was caused by other mechanisms than in otherwise healthy subjects

There is no certain explanation for the unexpected peaks in serum iron The time relationship to the rejection episodes is notable but of course does not prove a connection It has been claimed that cortisone can release iron from the reticuloendothelial system to plasma in patients with chronic inflammation (11) but no study has been made with such high doses as those given here It should be noted that 4 of the 5 rejection episodes that were not followed by a serum iron peak were treated with a lower cortisone dose than the rest

Local irradiation was given in all rejection treatment *The effect of this on serum iron is unknown* The transplant rejection is in itself by nature an inflammatory reaction and should if anything cause a fall in serum iron levels

In conclusion acute infection was shown to produce elevation in serum ferritin levels even in patients on immunosuppressive therapy The ferritin reaction was shown to start within 48 hours of the initial symptoms of infectious disease (temperature rise) The peak ferritin value was reached within a week except in 2 cases with very high ferritin values The fall after the peak was much faster than in patients previously studied The plasma half disappearance time for ferritin in the extreme case was as short as 1 5 days

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REFERENCES

1 Bentley D P & Williams P Serum ferritin concentration as an index of iron stores in rheumatoid arthritis J Clin Pathol 27 786 1974
2 Birgegård G Hallgren R Killander A Stromberg A Venge P & Wide L Serum ferritin during infection Scand J Haematol 21 333 1978
3 Bolton A E & Hunter W M The labelling of proteins to high specific radioactivities by conjugation to a 125I-containing alkylating agent Biochem J 133 529 1973
4 Cook J D Lipschitz D A Laughton Ch Miles E M & Finch C A Serum ferritin as a measure of iron stores in normal subjects Am J Clin Nutr 27 681 1974
5 Elin R J Wolff S M & Finch C A Effect of induced fever on serum iron and ferritin concentrations in man Blood 49 147 1977
6 Halliday J W Gera K L & Powell L W A solid phase radioimmunoassay for serum ferritin Clin Chim Acta 58 207 1975
7 Leyland M J Ganguli P C Blower D & Delamore I N Immunoradiometric assay for ferritin in *human serum Scand J Haematol 14 385 1975*
8 Lizana J & Hellsing K Polymer enhancement of automated immunological nephelometric analysis as illustrated by determination of urinary albumin Clin Chem 20 415 1974
9 Richterich R In Clinical chemistry theory and practice Academic Press New York 1969
10 Siimes M A Addiego J E & Dallman P R Ferritin in serum diagnosis of iron deficiency and iron overload in infants and children Blood 43 581 1974
11 Strandberg O The influence of corticosteroid therapy on hematological values Acta Med Scand (Suppl) 454 127 1966
12 *Walters G O Jacobs A Worwood M* Trevett D & Thomson W Iron absorption in normal subjects and patients with idiopathic haemochromatosis relationship with serum ferritin concentration Gut 16 188 1975
13 Wide L & Birgegård G A solid phase radioimmunoassay method for ferritin in serum using 125I labelled ferritin Ups J Med Sci 82 15 1977

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Increased Red Cell Content of Zn^{2+} in Essential Hypertension

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ABSTRACT The zinc content in plasma as well as in erythrocytes was studied in 61 patients with untreated essential hypertension They displayed an intraerythrocytic mean value of Zn^{2+} of 128 2 μmoles/l intracellular water which is significantly higher than the corresponding mean of *101 5 μmoles/l from 42* normal persons In plasma, on the other hand, the mean zinc value of the hypertensive persons was 15 2 μmoles/l plasma i e well within the normal range of plasma Zn^{2+} Possible mechanisms underlying the elevated red cell zinc level in hypertension are discussed

Key words hypertension zinc erythrocytes

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The biological role of the zinc ion has long been obscure in medicine (12) An increasing number of enzymes have been found to require Zn^{2} for their activities (4 11 13) The discovery by Barnes and Moynahan (2) that zinc deficiency is the molecular basis for acrodermatitis enteropathica was an important step forward from both the diagnostic and the therapeutic point of view Recently a patient was described with acrodermatitis enteropathica complicated by hypertension The acrodermatitis healed completely on zinc therapy Simultaneously the hypertension was controlled with a gradually decreased antihypertensive drug dosage Therefore a possible role of zinc in the pathogenesis of hypertension was suggested (1)

Bierenbaum et al (3) studied the possible relationship between exogenous zinc and cadmium in drinking water and the zinc and cadmium levels in serum as well as the blood pressure (BP) level The writers proposed that an inverse ratio of zinc/cadmium could influence the BP In an attempt to further elucidate the possible role of zinc in hypertension we have extended few earlier studies on zinc in serum to comprise the intracellular levels of zinc as measured in the human erythrocyte

PATIENTS

Sixty-one patients age range 30–69 years (mean 49 1) 29 men and 32 women with untreated essential hypertension were examined Physical examination laboratory tests and in some cases i v pyelography and/or renal angiography did not show any evidence of secondary hypertension

The criterion for the diagnosis of hypertension was a diastolic supine BP of ≥105 mmHg after 10 min rest on at least two occasions The range was 105–130 mmHg (mean 112 3±7 6) For comparison samples were drawn from 42 healthy normotensive volunteers age range 18–50 years (mean 41 3) 27 men and 15 women recruited from the same area around the hospital All had a diastolic pressure of ≤90 mmHg

Haematological parameters including the corpuscular constants were determined in the Hemalog 8 instrument (Technicon) in all of the patients studied and were in all respects within the normal limits adopted for this laboratory There was no reason to suspect lead exposure in any of the patients

SAMPLE COLLECTION AND ANALYSIS

Whole blood (about 15 ml) was obtained from each person *during the day between 0900 and 1630 hours* The blood was drawn by venipuncture into plastic syringes and prerinsed polypropylene tubes to which 20 μl of a sodium heparin solution (1000 USP units/ml) was added The samples were immediately chilled in ice water The entire system (including the heparin solutions) was checked for contamination with zinc *Only negligible (<0 1 μmol/l)* quantities of zinc were added to the blood specimen by the heparin No metal contamination by the needles syringes or propylene tubes could be detected The plasma was removed as well as the buffy coat layer The red cells

A preliminary report of this paper was presented at the VIII World Congress of Cardiology in Tokyo 1978 (6)

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were washed twice in an isotonic medium containing 130 mM NaCl and 25 mM KCl. Intracellular volume was determined for the packed red cells by estimation of wet and dry weight. A correction was made for saline trapping with the ^{131}I albumin method as described by Fortier et al (5).

The packed erythrocytes were haemolysed by adding 0.5 ml distilled water to 1.5 ml packed cells. Twenty microliters of a saponine solution (2 g of saponine in 10 ml of distilled water) were added to the blood water mixture. The solution was thoroughly mixed and rapidly frozen at −20°C in a dry ice-ethanol solution. The frozen material was either thawed immediately or kept frozen for at most 14 days before analysis.

During thawing 0.5 ml of 50 (w/v) trichloroacetic acid were added giving a tot l volume of 2.5 ml. The mixture was centrifuged d th clear supernatant was analysed for Zn^{2+}.

Zn^{2+} was dete n l b th in pl ma and in the haemolysate by a t h t ution of 1 10 for plasma and 1 100 for the superna btained with 6% (w/v) of n butanol. Analyses we e performed by atomic absorption emission spectrophot n etry h the IL353 instrument and expressed in µm s/l r and intracellular water respectively. Samples r t nely drawn for haematological examin a ega Hb red cell count and haematocrit as wel as the orpus ar constants.

To study a possible rel nship b een heparin and zinc ions and a possi le i uence of heparin on the distribution of zinc i s be een the int cellular and extracellular compartments one serie f xperiments was carried out in the following way. Bl d samples were from two normals in o tu es without any addi well as in tubes cont ning heparin as described sera and pl smas were parated by centrifugation subdivided into si one m ractions. Exogenous zinc ns were added to ea h portion in increasing amounts as internal standard as shown in the ordinate of Fig 1. The zinc content of the plasmas and sera was determined according to the procedure above.

RESULTS

The mean content (±S.D.) of Zn^{2+} in the erythrocytes of 42 healthy normals was 101.5±16.5 and of the 61 patients with essential hypertension 128.2±17.7 ($p<0.001$). It is apparent that the hypertensive patients display a significant increase of their intraerythrocytic zinc concentration compared with the normal individuals. They had, on the other hand, an entirely normal plasma zinc value (mean 15.2 µmol/l ± 3.9), i.e. well within the normal range adopted for this laboratory (11–18 µmol/l). There was no significant difference as regards intraerythrocytic zinc content between the sexes. This observation was valid for both the hypertensive patients and the normals. Linear regression analysis failed to show any correlation between the intraerythrocytic zinc levels and the systolic ($r=0.39$) as well as the diastolic ($r=0.16$) BP. The haematological parameters of the patients studied were in all respects within the normal limits.

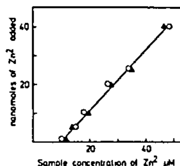


Fig. 1. Effect of exogenous zinc ions added to sera (○) and plasmas (▲) from two normal persons.

Fig. 1 clearly demonstrates that the zinc determination is not influenced by the heparin as examined with exogenous zinc as internal standard. Furthermore, it can be concluded that the zinc distribution between serum and the intraerythrocytic fluid is unchanged in the presence of heparin since there is a linearity of the plasma curve similar to the serum curve.

DISCUSSION

We report for the first time a significant increase in intraerythrocytic zinc in connection with essential hypertension. At the same time the lack of difference in the plasma zinc level between normal and hypertensive individuals is noteworthy.

Elevated erythrocyte zinc protoporphyrin is indicative of impaired heme biosynthesis caused by lead poisoning or iron deficiency (8). Since zinc protoporphyrin is located almost exclusively in the erythrocytes, an artefactually increased intracellular zinc concentration could theoretically occur in cases of iron deficiency or lead poisoning. This could be ruled out, however, due to normal corpuscular constants. No anemia appeared in any of the patients participating in this investigation although no specific test for possible lead poisoning was carried out. Anamnestically there was no reason to suspect lead poisoning in any of the patients.

The correlation between the zinc serum level and BP has been the subject of some recent studies. Bierenbaum et al (3) surveyed two populations as regards the content of trace metals in their drinking water, corresponding serum levels and BP. They

found a reciprocal relationship between the groups studied so that the persons having soft drinking water displayed a significantly higher serum zinc level and lower BP than those having hard drinking water The relationship between the latter s higher BP and lower serum zinc level was not further explored Also Åberg et al (1) claimed that it was not possible to find a causal relationship between zinc deficiency and hypertension in the patient pre sented by them The explanation for the increased intraerythrocytic Zn^{2+} concentration in essential hypertension is obscure However it is well known that the erythrocyte like kidney tissue contains a high amount of carbonic anhydrase (7) This is a metallo-enzyme and it requires Zn^{2+} in its active center Thus Zn^{2+} is a co-factor of carbonic anhy drase In contrast antihypertensive agents like thiazides act antagonistically to this enzyme (9) An attractive hypothesis would then be that if the same conditions are valid for the kidney tubular cells as for the erythrocytes an increased intracel lular zinc concentration favours the carbonic anhydrase activity and thereby constitutes an early step in the development of hypertension Another possibility not necessarily excluding the first one but instead coexisting and working synergistically might be that the zinc ions are inhibitors of the diesterase (4) thereby increasing the intracellular level of cyclic AMP Cyclic AMP is the second messenger in adrenergic stimulation and zinc dis plays and adrenergic effect not via the adenyl cyclase enzyme system but instead acting uniquely on this diesterase A prerequisite is of course that the normal intracellular concentration of zinc is not maximally inhibitory for the diesterase in vivo but instead an additional inhibition may be achieved by a further increase of the intracellular zinc ions

In this context it is interesting to note the recent report by Perry et al (10) who indeed found an increased zinc concentration in kidney tissue of rats made hypertensive by cadmium feeding

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REFERENCES

1 Åberg H Michaelsson G & Walldius G Hyper tension in a patient with acrodermatitis enteropathica Acta Paediatr Scand 65 757 1976
2 Barnes P M & Moynahan E J Zinc deficiency in acrodermatitis enteropathica Multiple dietary intol erance treated with synthetic diet. Proc R Soc Med 66 327 1973
3 Bierenbaum M Fleischman J Dunn J & Arnold J Possible toxic water factor in coronary heart-dis ease Lancet 1 1008 1975
4 Chvapil M New aspects in the biological role of zinc a stabilizer of macromolecules and biological membranes Life Sci 13 1041 1973
5 Fortier N Snyder M Palek J & Weiss E Effect of propranolol on human erythrocytes J Lab Clin Med 89 41 1977
6 Frithz G & Ronquist G Increased red cell content of Zn^{2} in essential hypertension Abstract 933 VIII World Congress of Cardiology Tokyo 1978
7 Harper H A In Review of Physiological chemistry pp 221 Blackwell Scientific Publishers Oxford and Edinburgh 1969
8 Lamola A A & Yamane T Zinc protoporphyrin in the erythrocytes of patients with lead intoxication and iron deficiency anemia Science 186 936 1974
9 Maren Th Carbonic anhydrase chemistry physiology and inhibition Physiol Rev 47 525 1967
10 Perry M Erlangen M & Perry F Elevated sys tolic pressure following chronic low level cadmium feeding Am J Phys 232 (2) H 114 1977
11 Sadler P Zinc in enzymes Nature 262 258 1976
12 Vallee B L Zinc biochemistry a perspective Trends in Biochemical Sciences 1 88 1976
13 Weismann K The biological role of zinc in normal and pathological conditions A survey Dan Med Bull 23 1-6 1976

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Resolution of Renal Amyloidosis Secondary to Rheumatoid Arthritis

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STRACT A patient with seronegative rheuma arthritis developed a nephrotic syndrome ological examination of renal biopsy dis d moderate amyloidosis Ultrastructurally the erular amyloid deposits were seen to be located within the mesangium and subepithelially in the pheral capillaries The patient was treated with nisone and cyclophosphamide for two years The rotic syndrome remitted and a follow up biopsy ed almost total disappearance of Congo red posi amyloid substance Electron microscopy showed dant finely granular material but only small unts of fibrillar amyloid in the mesangial regions intramembranous lucent areas containing few oid fibrils but no subepithelial deposits in the heral capillaries We conclude that the mesan amyloid substance was degraded to granular rial and that the subepithelial amyloid deposits resolved by mechanisms similar to those in d in the resolution of subepithelial immune lex deposits, i e through slow washing out and poration into the basement membrane

words amyloidosis rheumatoid arthritis
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i after successful eradication of the underlying se regression of secondary renal amyloidosis is and has been demonstrated histologically in ases only (3 6 8 10 16) Renal amyloidosis dary to diseases less accessible to radical nent such as rheumatoid arthritis has been dered irreversible although some encouraging s have been obtained in treatment of oidosis associated with juvenile rheumatoid itis with chlorambucil (13) One case of ogically proved regression of renal amyloi secondary to rheumatoid arthritis after treat with prednisone and cyclophosphamide has reported (8)

report a case with renal amyloidosis secondary to rheumatoid arthritis Re i i i nephrotic syndrome occurred after t ta ment with prednisone and cyclopho mide solution of renal amyloid deposit der n strated by Congo red staining on ligi n p and by electron microscopy

CASE REPORT

In 1970 a 45 year old nurse developed chr c polyarthritis affecting the joints of the hands wrists feet and knees Radiologically detectable joint erosions were observed in 1972 When checked again in 1975 they had extended considerably The tests for rheumatoid fac or were negative or weakly positive The antinuc ar anti body tests were moderately to highly positive from the beginning but the DNA antibody tests and the LE cell preparations were negative The patient was considered to be suffering from seronegative erosive rheumatoid arthritis with antinuclear antibodies Gold salt treatment was tried in 1971 but caused slight transient proteinuria

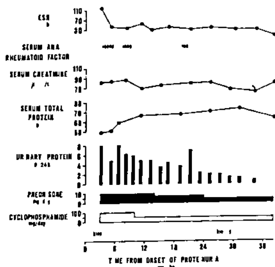


Fig 1 Summary of the clinical course after the onset of proteinuria

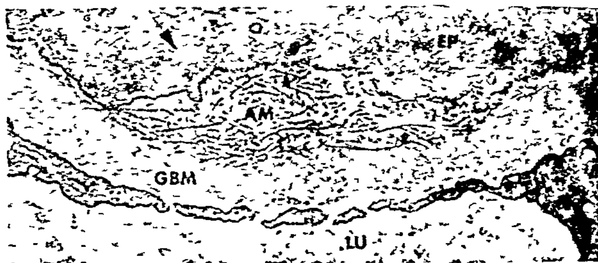


Fig 2 Initial biopsy. Electron micrograph of peripheral glomerular capillary wall showing subepithelial deposit of fibrillar amyloid (AM). Basement membrane (*GBM*) is intact. Condensation (arrow) of epithelial (*EP*) cytoplasm with loss of foot processes adjacent to the deposit may indicate local leakage of protein. *LU* = capillary lumen. ×33 000.

and was abandoned. Prednisone was given for short periods in 1972 and [illegible]. In July 1975 a mild nephrotic syndrome was observed [illegible] proteinuria amounting to 8 g/24 h. The patient was admitted to our hospital for assessment. Pertinent clinical data are summarized in Fig. 1.

The first renal tissue biopsy was performed three months after the onset of proteinuria. The biopsy showed amyloidosis as described below in the study of pathology. Additionally proliferative changes were observed and [illegible] with prednisone [illegible] mg/day and cyclophosphamide 1.0 mg/kg was started. The nephrotic syndrome disappeared and the laboratory parameters improved as shown in Fig. 1. The condition of the joints remained rather steady, allowing the patient to continue her work. A follow up biopsy was carried out in Nov 1977, 25 months after the initial biopsy, when proteinuria amounted to only 1.4 g/24 h.

PATHOLOGY

The renal biopsies were studied by light, electron and immunofluorescence microscopy using well described methods (14). Light microscopic examina-

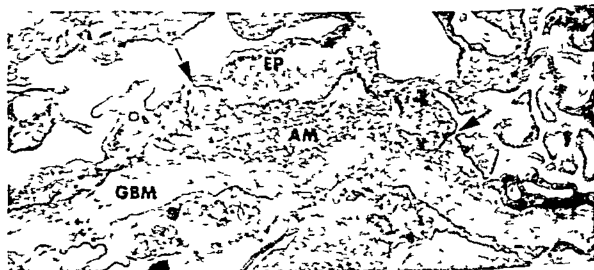


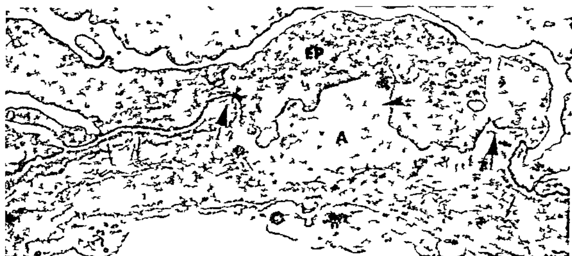
Fig 3 Initial biopsy. Electron micrograph of glomerular capillary wall showing subepithelial aggregate of amyloid fibrils (*AM*) extending up to the epithelial (*EP*) slit pore diaphragms (arrows). *GMB* = capillary basement membrane. ×18 000.



'g 4 Follow up b opsy Electron m crograph of glo-
erular mesangial depos t composed partly of f bnllar
mylo d (AM) and partly of f nely granular material (GR)
distinct amylo d f bnls (arrows) are present n the bor
r zone suggest ng a break-down of f bnls into granular
aterial ×30 000

on of the initial biopsy showed a moderate amount f Congo red positive amylo d depos ts in the omerular mesangial regions with typical apple reen b refringence when stud ed under polarized ght In addit on there were some glomerular pro-liferative changes includ ng focal epithel al cres-ents which are known to be sometimes associated with amylo dosis (20) Amylo d depos ts were also detected in the walls of some blood vessels and in the interst tium On electron m croscopy accumulations of typical non branching 80–100 Å thick amyloid fibrils were observed in the glomerular mesangium and segmentally in the peripheral cap l laries In the peripheral cap llaries the amylo d deposits were located mainly subep thel ally the underlying basement membrane (GBM) be ng intact (Fig 2) In this location the amyloid fibrils were often arranged in parallel bundles or tufts extend ng up to the epithelial slit diaphragms (Fig 3) No GBM l ke material was present on the epithel al s de of the amylo d depos ts No granular electron dense depos ts suggesting e g lupus nephritis or gold nephropathy were seen In sections stained for immunofluorescence deposits of IgG and C3 were seen n the glomerular capillary walls and the mesang um

In the follow up biopsy almost no Congo red positive material was seen on l ght m croscopy Some glomeruli showed sclerosed areas and capsular adhes ons On electron microscopy the mesangial deposits of f brillar amyloid had decreased considerably in size and seemed to have been partly replaced by deposits of a finely granular material (F g 4) In the peripheral cap llaries the GBM was irregular due to indentations and intramembranous lucent areas of varying size which



F g 5 Follow up b opsy Electron m crograph of periph-eral cap llary wall Basement membrane s irregularly thickened w th project ons (arrows) surround ng large translucent area (A) wh ch contain remnants of amyloid f bnls (arrowhead) More d stin t amylo d f bnls are seen w th n he basement membrane (small arrows) N segmental loss of ep thel al (EP) foot processes w densat on of ep thelial cytoplasm ×16 000



Fig 6 Follow up biopsy Electron micrograph of glomerular capillary wall showing longitudinally and transversely sectioned amyloid fibrils (arrows) located within the basement membrane covered by an apparently newly formed layer of *GBM* (double arrow) Epithelial (*EP*) foot processes are normal *LU*=capillary lumen ×19000

often contained small amounts of amyloid fibrils (Figs 5 and 6) In many places however the lucent areas lacked all traces of amyloid fibrils (Fig 7) and were indistinguishable from highly resolved subepithelial immune complex deposits in membranous glomerulonephritis (15) and in poststreptococcal glomerulonephritis (14) (Fig 8 shown for comparison) No subepithelial deposits of amyloid were seen On immunofluorescence deposits of IgG and C3 were still seen in the follow up biopsy

DISCUSSION

The findings in the first biopsy meet the current histological and ultrastructural criteria of amyloid i e green birefringence on polarization microscopy after Congo red staining and non branching 80–100 Å thick fibrils on electron microscopy (?) In the follow up biopsy Congo red staining had turned practically negative while typical fibrillar amyloid was still detectable by electron microscopy The e findings show the superiority of electron microscopy to light microscopy in demonstrating such small amounts of glomerular amyloid and thereby in analyzing the morphological aspects of resolution of glomerular amyloidosis

Regression of amyloid deposits in the liver was first demonstrated by Waldenstrom in 1928 (17) and since then by others (4 12) Although remission of clinical signs of renal amylo dosis has been reported



Fig 7 Follow up biopsy Electron micrograph of peripheral capillary wall Basement membrane shows projections (arrowheads) deep epithelial indentations (arrows) and large translucent areas (*A*) The latter evidently represent residues of former subepithelial amyloid deposits Amyloid fibrils are almost absent Epithelial (*EP*) foot processes are broadened ×11000



Fig 8 Electron micrograph of resolving phase of immune complex glomerulonephritis (membranous glomerulonephritis) shown for comparison Intramembranous lucent areas (*A*) represent residues of former subepithelial immune complex deposits Compare with Figs 5–7 ×17000

In many cases histological evidence of resolution of renal amyloid deposits has been obtained in only seven cases (3 6 8 10 16) and only one of these cases had amyloidosis secondary to rheumatoid arthritis (8) It has been considered a prerequisite for regression of amyloidosis that the underlying disease is completely cured but since this is not possible today in the case of rheumatoid arthritis the cause of regression in our patient remains speculative Our patient was treated with prednisone and cyclophosphamide as was the one previously reported patient with resolved renal amyloidosis associated with rheumatoid arthritis (8) Encouraging clinical results have also been obtained with chlorambucil in amyloidosis secondary to juvenile rheumatoid arthritis (13) If these drugs exert an actual effect on the resolution process it seems more likely that they do so by reducing the activity of the rheumatic disease rather than by interfering with the formation of the amyloid substance itself since it has been shown that corticosteroids and antimetabolites have no suppressive effect on experimental amyloidosis (1 9)

Electron microscopic studies were included in only two previous reports on resolving renal amyloidosis They showed that basement membrane like material was formed over subepithelial amyloid deposits (3 6) this picture being considered to have features analogous to the changes in membranous glomerulonephritis (3) The ultrastructural findings in our case provide more detailed information on the mechanisms of the resolution process of amyloid deposits in the glomerulus Probably the deposition of new amyloid substance decreased or ceased after the first biopsy In the course of time the subepithelial amyloid deposits lose their fibrillar structure and electron density probably due to local degradation and slow washing out of the amyloid substance These deposits are filled out by epithelial indentations or become covered by new basement membrane material as a result of the natural turnover process of the membrane (18) This sequence of events resulting in an irregularly indented and mottled basement membrane is very similar to the resolution of subepithelial immune complex deposits in membranous glomerulonephritis (15) and in acute poststreptococcal glomerulonephritis (14) These findings indicate that this reparative process also works with material other than immune complexes

In one of the two previous ultrastructural studies the mesangial amyloid deposits in the follow up biopsy were described as electron dense with crumbly fibrils and were designated inactive but their Congo red stainability was not commented upon (6) In our case replacement of fibrillar material by granular material was observed in the mesangial deposits This change may indicate a breakdown process and is presumably the process that makes the amyloid substance Congo red negative

We may speculate that the subepithelial deposits of amyloid and of immune complexes also have similarities in morphogenesis i e that amyloid deposits are derived from circulating precursors rather than formed in situ If this were the case the question arises which are the mechanisms by which circulating precursors such as serum protein S (19) become trapped and polymerize to form

in the subepithelial space On the basis of molecular weight alone (approx 12 000 daltons) protein SAA monomers should be able to pass not only through the GBM but also through the epithelial slit diaphragms (7) Lambda light chains pass readily through the glomerular filter and are probably precursors of the Congo red positive material that is sometimes seen in the tubular casts of myeloma kidney (5) Thus other factors e g molecular charge (11), must be implicated in the process of trapping and aggregating of amyloid precursor molecules such as SAA on the outside of the GBM

The most distinct qualitative alterations of the amyloid deposits in our case signifying a resolution *process occurred in the peripheral capillaries* Light microscopy is certainly an insensitive method for detecting such regressive changes because subepithelial deposits are too small to be visualized in Congo red stained paraffin sections

This study shows that forces are available for removing even the inert amyloid substance from the kidney and suggests that also rheumatoid arthritis should be treated as vigorously as possibly It sheds new light on the dynamics of amyloid deposits in the glomerulus and provides a means for assessing the evolutionary or reparatory state of resolving renal amyloidosis It suggests that all the rare cases f remission of renal amyloidosis should be ex-ined by electron microscopy in order to elucidate the nature of the resolving process further and eventually to find a future treatment of the disease

REFERENCES

1 Cohen A S Calkins E & Mullinax P F Studies in experimental amyloidosis Arch Intern Med 110 569 1962
2 Cohen A S Cathcart E S & Skinner M Amyloidosis Current trends in its investigation Arthritis Rheum 21 153 1978
3 Dikman S H Kahn Th Gribetz D & Churg J Resolution of renal amyloidosis Am J Med 63 430 1977
4 Fitchen J H Amyloidosis and granulomatous ileocolitis Regression after surgical removal of the involved bowel N Engl J Med 292 352 1975
5 Friman C Tornroth T & Wegelius O I myeloma associated with multiple extramedu amyloid-containing tumours and amyloid casts in t renal tubules Ann Clin Res 2 161 1970
6 v Gise H Helmchen U Mikeler E Bruning L Walther Ch Christ H Mackensen S & Bohle A Correlations between the morphological and clinic findings in a patient recovering from second generalised amyloidosis with renal involvement Vi chows Arch A 379 119 1978
7 Graham R C & Karnovsky M J Glome permeability Ultrastructural and cytochemical stu ies using peroxidases as protein tracers J Exp M 124 1123 1966
8 Hajzok O Tomik F & Hajzokova M Amyk dosis in rheumatoid arthritis A study of 48 hist logically confirmed cases Z Rheumatol 35 3? 1976
9 Hardt F Acceleration of casein-induced amyk dosis in mice by immunosuppressive agents Ac Pathol Microbiol Scand (A) 79 61 1971
10 Kuhlback B & Wegelius O Secondary amy dosis A study of clinical and pathological finding Acta Med Scand 180 737 1966
11 Latta H Johnston W H & Stanley T M *Sialoglycoproteins and filtration barriers in t* glomerular capillary wall J Ultrastruct Res 51 3? 1975
12 Lowenstein J & Gallo G Remission of the n phrotic syndrome in renal amyloidosis N Engl J M 282 128 1970
13 Schnitzer T J & Ansell B M Amyloid in juvenile chronic polyarthritis Arthritis Rheu (Suppl) 2 245 1977
14 Tornroth T The fate of subepithelial deposits acute poststreptococcal glomerulonephritis Lab I vest 35 461 1976
15 Tornroth T & Skrifvars B The development a resolution of glomerular basement membrane chan associated with subepithelial immune deposits A Pathol 79 219 1975
16 Triger D R & Joekes A M Renal amyloidos A fourteen year follow up *Q J Med 165 15 1973*
17 Waldenstrom H On the formation and disapp ance of amyloid in man Acta Chir Scand 63 1928
18 Walker F The origin turn-over and removal glomerular basement membrane J Pathol 110 ? 1973
19 Wegelius O & Pasternack A (eds) Amyloidosi ix Academic Press London 1976
20 Zollinger H U & Mihatsch M J Renal patholo in biopsy p 385 Springer Verlag Berlin 1978